

UNIVERSIDADE FEDERAL DO PARANÁ
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ECOLOGIA ALIMENTAR E CONECTIVIDADE GENÉTICA DE *CHAETODON*
STRIATUS EM UM GRADIENTE GEOGRÁFICO

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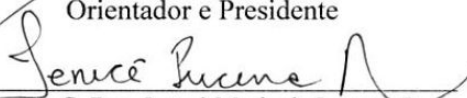
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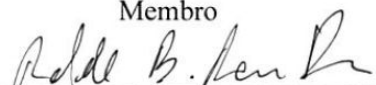
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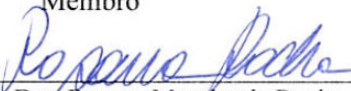
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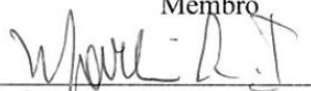
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Resumo

Conhecer a ecologia alimentar de uma espécie é um aspecto importante para compreender sua biologia. A relação entre disponibilidade, qualidade do alimento e estado fisiológico reflete na distribuição e abundância de uma dada espécie, e terá influência direta no comportamento alimentar e tipo de presas. A relação entre plasticidade alimentar e fluxo gênico pode levar as populações das extremidades da distribuição a se diferenciarem e possivelmente se adaptarem aos recursos disponíveis. O fato de algumas espécies possuírem ampla distribuição faz com que elas se tornem excelentes modelos para testar fluxo gênico e as diferenças entre populações que habitam ambientes com diferentes atributos ecológicos. O presente estudo é o primeiro a comparar três abordagens complementares para compreender o comportamento alimentar de peixes-borboleta generalistas: forrageamento, conteúdo estomacal e condição nutricional. Além disso, foi também estimada a conectividade genética com o uso de marcadores moleculares. Enquanto a maioria dos estudos com peixes-borboleta já realizados no Indo-Pacífico, no Caribe e no Mar Vermelho, pouco se sabe sobre as espécies que habitam as águas que circundam o Novo Mundo. O subgênero *Chaetodon* é formado por dois pares de espécies: *Chaetodon striatus* e *C. capistratus* e o clado irmão *C. ocellatus* e *C. humeralis*. A espécie foco desse trabalho é *C. striatus*, a qual é amplamente distribuída no Atlântico Ocidental, habitando desde os recifes de coral no Caribe até os recifes rochosos no sul da costa brasileira. Essa ampla distribuição inclui diferentes províncias biogeográficas e barreiras (ex. a foz do Rio Amazonas), assim como fatores bióticos e abióticos (ex. tipo de recife e temperatura da água). O trabalho de campo foi realizado em oito localidades (Porto Rico, Tamandaré, Salvador, Abrolhos, Guarapari, Ilha de Trindade, Arraial do Cabo e Florianópolis). Não foram encontradas diferenças entre tamanho de corpo e densidade nas populações amostradas. No entanto, foram encontradas diferenças na taxa de mordidas entre os indivíduos das diferentes localidades, porém constatou-se que as populações dos extremos são similares estatisticamente. Esses resultados contrariam os resultados esperados, segundo as quais as espécies que se alimentam de invertebrados sésseis teriam baixa abundância e baixa taxa de mordida nas localidades mais distantes dos trópicos. 55 itens foram encontrados no conteúdo estomacal, incluindo anelídeos, artrópodes, equinodermatas, cnidários e poliquetas. Os itens mais frequentes foram poliquetas e cnidários. Quando *C. striatus* co-ocorre com sua espécie-irmã no Caribe, foi visto que existem diferenças nas taxas de mordidas e abundância entre as espécies. Enquanto a maior parte das mordidas de *C. striatus* ocorreu na matriz de algas epilíticas e na areia, *C. capistratus* mordeu mais em corais, porém sem apresentar seletividade para esse item. *Chaetodon humeralis* é a única espécie de peixe-borboleta presente

no Pacífico Oriental Tropical e pouco se conhece sobre seu comportamento alimentar. Verificou-se que essa espécie exibe uma ampla gama de itens alimentares, incluindo também algas (16%) em sua dieta. Em geral, mesmo apresentando adaptações para incluir em sua dieta cnidários com defesas mecânicas e/ou químicas, todas as espécies estudadas podem ser consideradas generalistas quanto a dieta e forrageamento. A análise filogeográfica de *C. striatus* indica ausência de estruturação populacional e todas as populações estudadas possuem a mesma diversidade genética. Mais ainda, essa espécie passou por uma expansão populacional de 80-100 mil anos, possivelmente devido às mudanças climáticas do Pleistoceno, quando aumentou a área da plataforma rasa.

Palavras-chave: peixes-borboleta, peixes recifais, Atlântico Ocidental, fluxo gênico, dieta, forrageamento

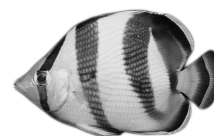
Abstract

The feeding ecology of a species is an important aspect of its biology. The relationship between food availability, food quality and physiological state will likely reflect in its distribution and abundance and also will directly influence its feeding behavior and prey types. The relationship between feeding plasticity and gene flow can lead to differentiation on the population at the extremes of ranges and possibly adapt to resource availability. The fact that some species have large distribution ranges made them excellent models to test differences between ecological differences and gene flow between populations. The present study is the first to compare three complementary approaches to understand the feeding behavior of generalist butterflyfishes: foraging mode, stomach contents and nutritional condition. Also, genetic connectivity was estimated using molecular markers. While the majority of studies with butterflyfishes have been carried out in the Indo Pacific, in the Caribbean and in the Red Sea, little is known about the New World's species. The subgenus *Chaetodon* is formed by two species pairs: *Chaetodon striatus* and *C. capistratus* and its sister clade *C. ocellatus* and *C. humeralis*. The focus of this research was *C. striatus*, a widely distributed species in the Western Atlantic Ocean, inhabiting from coral reefs in the Caribbean to the rocky reefs on the southern Brazilian coast. This large distribution range includes different biogeographic provinces and barriers (e.g. outflow of Orinoco and Amazon rivers), as well as biotic and abiotic differences (e.g. reef type and water temperature). Field work was conducted in eight localities (Puerto Rico, Tamandaré, Salvador, Abrolhos, Guarapari, Ilha de Trindade, Arraial do Cabo and Florianópolis). There were no major differences in body size and density among populations. However, differences in feeding rates among populations were detected but it did not follow the expectation that fishes that feed on sessile invertebrates have lower abundance and feeding rates out of the tropics. Electivity indices did not show selectivity patterns among sites, suggesting that *Chaetodon striatus* has high foraging plasticity. Fifty-three different items were found in the stomach contents, including Annelida, Arthropoda, Echinodermata, Cnidaria and Polychaeta. The main items consumed were Polychaeta and Cnidaria. When co-occurring with its sister-species in the Caribbean, differences on feeding rates and abundance were detected. While *Chaetodon striatus* took more bites on the epilithic algal matrix and sand microhabitats, *C. capistratus* took more bites on octocorals and hard corals, although not presenting selectivity patterns. The threebanded butterflyfish, *Chaetodon humeralis* is the only species of this genus in the Tropical Eastern Pacific and little is known about its feeding behaviour. This species exhibited a high diet breadth in the Sea of Cortez, even including 16% algae in its diet. In general, despite having adaptations to include mechanically or chemically defended

cnidarians in their diets, all studied species could be considered generalists in their diet and foraging behaviour. Phylogeographic analyses indicated that there is a lack of population structure of *C. striatus*, and all populations have the same genetic diversity. Also, this species have had a recent population expansion around 80-100 thousand years, probably due to the Pleistocene climate changes associated with the enlargement of coastal shelf areas.

Keywords: butterflyfish, reef fish, Western Atlantic, gene flow, diet, forage.

Introdução Geral



A família Chaetodontidae

Os peixes da família Chaetodontidae estão entre as espécies mais admiradas nos ambientes recifais devido a diversidade e exuberância de cores. A maioria das espécies é conhecida popularmente como peixes-borboleta. Com o corpo lateralmente achatado, em forma de disco, chamam atenção com aproximadamente 130 espécies distribuídas em 11 gêneros e presentes em todos os oceanos tropicais e subtropicais, sendo que 90% vivem no Oceano Pacífico (Kuitert, 2002). Além disso, algumas espécies conseguem habitar águas estuarinas (Nelson, 2006). Muitas espécies possuem uma faixa preta sobre os olhos e também um ocelo na porção posterior do corpo, ambas as características para despistar os predadores (Nelson, 2006).

As espécies de peixes-borboleta geralmente possuem sua distribuição geográfica relacionada com a temperatura da água e a disponibilidade do substrato. Poucas são as espécies que conseguem habitar águas subtropicais/temperadas, onde a temperatura superficial da água pode atingir valores inferiores a 15°C (Kuitert, 2002). Com isso, a área de distribuição das espécies varia bastante, sendo que algumas espécies são restritas a pequenas regiões, enquanto outras espécies têm ampla distribuição (Kuitert, 2002). Além disso, a profundidade onde ocorrem também varia, tanto entre as espécies quanto ao longo da distribuição de uma mesma espécie (Kuitert, 2002).

Os peixes-borboleta geralmente vivem em pares monogâmicos por anos e cada par habita uma determinada área no recife, na qual defendem de outros pares da mesma espécie (Driscoll e Driscoll, 1988). Além disso, essas espécies são conhecidas por viverem intimamente associadas ao substrato uma vez que consomem uma grande variedade de presas bentônicas (Randall, 1967; Pratchett, 2005). Esse comportamento alimentar é devido a especialização da mandíbula e dentes, na qual permite que os peixes-borboleta consumam diferentes itens alimentares (Motta, 1988; 1989).

Tipicamente, as espécies dessa família são classificadas quanto a sua alimentação como: coralívoras obrigatórios, coralívoras facultativos e generalistas. Ainda, existem espécies que se alimentam no plâncton (Sazima e Sazima, 2001; Pratchett, 2005). A maior parte dos estudos de forrageamento de peixes-borboleta foi realizada no Oceano Pacífico, com espécies coralívoras, justamente porque nesta região está concentrada a maior diversidade de espécies (Kuitert, 2002; Pratchett, 2005). Estudos mostram que a dieta de algumas espécies não-coralívoras obrigatórias do Pacífico e do Caribe, inclui antozoários, poliquetas e pequenos crustáceos (Randall, 1967; Birkeland e Neudecker, 1981; Pratchett, 2005).

Assim como diversas espécies recifais, os peixes-borboletas possuem movimentação limitada durante a fase adulta, de modo que a principal oportunidade de dispersão e expansão da distribuição ocorre durante a relativamente curta fase larval (Leis, 1991). Os peixes recifais, de uma maneira geral, apresentam dois tipos principais de desova: a desova demersal, na qual os ovos permanecem no fundo até o momento da eclosão e posteriormente as larvas “derivam” no plâncton e dispersam; e a desova pelágica, na qual os ovos são lançados diretamente na coluna da água, e dessa forma, permanecem mais tempo no plâncton, teoricamente possuindo maior capacidade de dispersão (Leis, 1991). Apesar das larvas de peixes recifais terem potencial para dispersão em longas distâncias (e.g. Scheltema, 1986), nos últimos anos houve um acúmulo de evidências de que a dispersão pode ser limitada (Jones et al., 1999, Swearer et al., 1999; Booth e Oveden, 2000; Cowen et al., 2006), isto é, o auto-recrutamento de peixes recifais é significativo (Jones et al., 1999).

Os Chaetodontidae apresentam o tipo de desova pelágico, na qual os ovos são soltos na coluna de água. O tempo de permanência dos ovos e larvas na coluna da água é entre 32- 52 dias (Booth e Parkinson, 2011; Leis e Yerman, 2012).

O subgênero *Chaetodon*

Esse subgênero inclui sete espécies, na qual três ocorrem no Oceano Atlântico Oriental (*Chaetodon robustus*, *C. hoefleri*, *C. marleyi*), três no Oceano Atlântico Ocidental (*C. striatus*, *C. capistratus* e *C. ocellatus*) e uma no Oceano Pacífico Oriental (*C. humeralis*) (Kuitert, 2002). Em filogenias recentes, esse agrupamento não resultou em um grupo monofilético (Fessler e Westneat, 2009; Bellwood et al., 2010), porém as últimas quatro espécies formam um clado único, onde *C. striatus* é espécie irmã de *C. capistratus* e *C. ocellatus* espécie irmã de *C. humeralis* (Bellwood et al., 2010). Essas últimas espécies foram separadas com o fechamento do Istmo do Panamá, há aproximadamente 3.1–3.5 milhões de anos atrás (Ma) (Lessios, 2001). Nesse estudo, consideraremos o subgênero *Chaetodon* formado por essas quatro espécies monofilético, conforme a figura 1.

O peixe-borboleta listrado - *Chaetodon striatus*

O peixe-borboleta listrado *Chaetodon striatus* (Fig. 2) é a espécie de peixe-borboleta com a mais ampla distribuição no Oceano Atlântico Ocidental, desde Nova Jersey, nos Estados Unidos, até o estado de Santa Catarina, Brasil (Carvalho Filho, 1999). Essa ampla distribuição abrange diferentes províncias biogeográficas, gradientes de temperatura (17–30°C) e diferentes sistemas recifais (biogênicos e abiogênicos) (Floeter et al., 2008).

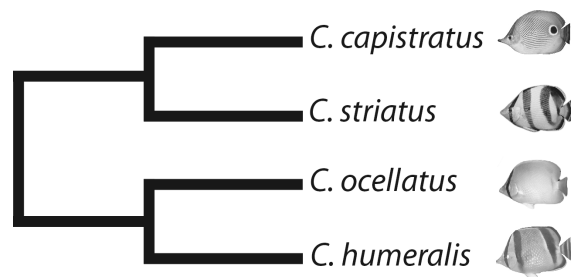


Figura 1. Relações filogenéticas entre as espécies do subgênero *Chaetodon* (Bellwood et al., 2010).

Chaetodon striatus é abundante e encontrado desde recifes rasos até áreas mais profundas, até 60 m (R Macieira, com pessoal). Os indivíduos juvenis possuem um ocelo na porção posterior superior do corpo, que gradualmente desaparece com o crescimento, estando totalmente ausente na fase adulta (Kuitert, 2002). Essa espécie atinge tamanho de até 17 cm (observação pessoal), maior do que descrito na bibliografia (15 cm, Kuitert 2002; 15,2 cm Human e Deloach 2002; 15 cm Carvalho-Filho 1999)

Apesar da ampla distribuição e abundância relativamente grande, os únicos registros de conteúdo estomacal dessa espécie foram publicados por Randall (1967) e Dubiaski-Silva et al. (2008) para indivíduos em Porto Rico e Ilhas Virgens, no Caribe e em Bombinhas, Brasil, respectivamente. Ambos apontam que o conteúdo estomacal de indivíduos amostrados é constituído principalmente de poliquetas e antozoários não escleractíneos. Alguns estudos abordam a observação em campo de microhabitats utilizados para forrageamento, porém em escala local (Bonaldo et al., 2005) assim como eventos esporádicos de alimentação no plâncton (Sazima e Sazima, 2001). Contudo, estudar essa espécie com uma ótica macroecológica é fundamental para compreender como essa espécie se distribui em aproximadamente 10.000km.



Figura 2. Peixe-borboleta listrado, *Chaetodon striatus*.

A costa brasileira

A extensa costa brasileira possui ambientes com características distintas, sendo que no nordeste a formação de arenito possui uma diversidade na cobertura bentônica, incluindo corais e algas calcárias, enquanto no sudeste-sul a formação dos recifes é rochosa. Essa mudança na estrutura das comunidades recifais (Maida e Ferreira, 1997; Floeter et al., 2001) é acompanhada pela diferença na temperatura mínima da água, que pode ser inferior a 18°C em certas épocas na porção sudeste-sul. Devido ao efeito da baixa temperatura da água para organismos de origem tropical, algumas espécies têm baixa abundância, o que se reflete na pouca disponibilidade de larvas, e por sua vez na restrição da distribuição, i.e. limite sul da distribuição. McBride e Able (1998) realizaram um estudo que mostra exatamente isso, porém para o Hemisfério Norte. Eles verificaram que juvenis de três espécies de peixe-borboleta do gênero *Chaetodon* dispersam para as águas temperadas (temperatura $\leq 15^{\circ}\text{C}$), porém morrem de hipotermia no inverno.

Na costa brasileira, a temperatura da água é estabelecida principalmente por duas correntes marinhas: a Corrente do Brasil, vindo do norte com águas quentes, e a Corrente das Malvinas, vindo do sul com águas geladas. Santos et al. (2006) demonstram que apesar da capacidade de dispersão durante o período pelágico, a temperatura das diferentes correntes marítimas age como principal barreira entre a porção nordeste e sudeste-sul, e apesar de espécimes de Pescadinha (*Macrodon ancylodon*) serem morfologicamente indistinguíveis entre essas regiões, dados moleculares separam claramente em grupos distintos.

O sul do Brasil é considerado limite de distribuição para espécies tropicais recifais também porque ao sul de Santa Catarina existe uma lacuna de recifes rochosos por uma extensão de aproximadamente 600 km, ocorrendo novamente somente no Uruguai. Isso implica em uma longa distância sem substrato para as larvas assentarem, e quando somada à temperatura baixa da água no inverno, se torna fator limitante.

O Caribe

O Caribe é a região com a maior biodiversidade do Oceano Atlântico (Briggs, 2003; Floeter et al. 2008) . Os recifes de coral são exuberantes, com mais de 230 espécies de corais, sendo aproximadamente 120 espécies de corais escleractíneos (Reefbase, 2013; www.reefbase.org). Os ambientes recifais apresentam alta complexidade e são de formação biogênica. Outra característica dessa região é a temperatura da água, que possui

temperaturas elevadas durante todas estações do ano, devido à influência das correntes marinhas e da conformação continental. Além disso, em geral, nos ambientes recifais a água é transparente, chegando a ter mais de 25 m de visibilidade.

Estudos da composição da dieta

Observações de comportamento alimentar (Fig.3) podem ser utilizadas para estimar taxa de mordidas e sugerir quais itens compõem a dieta. Para algumas espécies é possível verificar os itens ingeridos através de observação em campo, como por exemplo, os coralívoros obrigatórios (Pratchett, 2007). Porém, para espécies generalistas, que procuram alimentos pequenos e de difícil visualização para o mergulhador, apenas a observação em campo não é suficiente para verificar os itens da dieta. No entanto, saber quais microhabitats estão sendo utilizados no forrageamento é interessante, uma vez que pode ser verificado se existe seletividade por microhabitats.

Pequenos invertebrados (ex. poliquetas e antozoários) vivem escondidos em microhabitats, como por exemplo, na matriz epilítica de algas (EAM), areia (ex. Harmelin-Vivien e Bouchon-Navaro 1983; Kramer et al., 2012) ou ainda sobre antozoários (ex. Gleibs et al., 1995; Nagelkerken et al., 2009). Existem poucos estudos que avaliam a atividade alimentar de espécies generalistas, pela dificuldade em identificar as presas. Por isso, unir essa metodologia de observação em campo com análise de conteúdo estomacal é fundamental para atingir o conhecimento global da dieta da espécie.

Condição nutricional RNA:DNA

Diversas espécies de peixes tropicais desenvolveram a habilidade de sobreviver em dietas de baixa caloria (ex. herbívoros, coralívoros) (Harmelin-Vivien, 2002; Floeter et al. 2004). Algas e animais sésseis (e.g. corais, esponjas) presentes nos ambientes recifais representam um recurso calórico de baixa qualidade e de difícil assimilação, muitas vezes devido a defesas químicas e/ou estruturais (Horn, 1989; Harmelin-Vivien, 2002).

Para suprir o duplo desafio da dieta de baixa caloria e difícil assimilação, os peixes que utilizam esses recursos devem se alimentar com mais frequência do que espécies de dieta carnívora para suprirem suas necessidades energéticas (Arrington et al., 2002), ou possuírem plasticidade alimentar suficiente para enriquecer a dieta com maior conteúdo protéico (Ferreira et al., 1998). Muitas dessas espécies possuem ampla distribuição, desde as regiões tropicais até águas sazonalmente mais frias nas regiões subtropicais (Floeter et al., 2008).

A temperatura superficial da água, a qualidade do alimento e o tamanho do corpo são considerados importantes determinantes para assimilação do alimento (Kooijman, 2000). Em baixas temperaturas, a taxa alimentar de espécies que utilizam recursos de baixo valor calórico diminui (Barneche et al., 2009) e elas podem vivenciar uma desproporção nos custos metabólicos, caso a taxa alimentar decline proporcionalmente mais do que as taxas metabólicas (Floeter et al., 2005). Quando comparado a espécies congêneras do Caribe e Pacífico que se alimentam predominantemente de corais escleractíneos (baixa qualidade calórica), o peixe-borboleta listrado, *Chaetodon striatus*, possui uma frequência alimentar menos intensa (Bonaldo et al., 2005), provavelmente devido à presença de invertebrados móveis (ricos em proteína) em sua dieta (Randall, 1967), o que representa um determinado grau de versatilidade alimentar.

Dessa maneira, espécies de ampla distribuição com dietas versáteis representam um modelo ideal para testar se a temperatura limita as dietas de baixa qualidade calórica, sendo que quanto menor for a temperatura, menor será a importância de itens de baixa qualidade calórica na dieta dessas espécies (Behrens e Lafferty, 2007). A análise da razão RNA-DNA pode indicar o sucesso fisiológico de uma espécie em relação à temperatura e dieta (Buckley e Szmant, 2004, Behrens e Lafferty, 2007). Essa razão reflete a capacidade de síntese de proteína do tecido e indica a condição nutricional e energética de um animal, de maneira que essa técnica pode ser empregada em uma grande diversidade de organismos (Dahlhoff e Menge, 1996, Buckley e Szmant, 2004). Através dessa técnica, Behrens e Lafferty (2007), em experimentos controlados, demonstraram que a espécie onívora de peixe recifal *Girella nigricans* teve uma melhora de 100% no desempenho em baixas temperaturas (12°C) quando itens de alto valor protéico foram adicionados a dieta.

Conectividade genética de populações

Para verificar a estruturação e conectividade entre as populações de *Chaetodon striatus*, foi realizado um estudo filogeográfico buscando compreender a atual distribuição dessa espécie. O conceito de filogeografia foi introduzido por Avise et al. (1987) para designar o estudo da distribuição da variabilidade genética de uma espécie em uma escala espacial e temporal. Os estudos filogeográficos têm por objetivo revelar a história evolutiva de uma linhagem, relacionando-a com sua distribuição geográfica, através, principalmente, das diferenças entre seqüências de DNA mitocondrial (mtDNA) (Avise, 2000). Análises de padrões filogeográficos permitem a verificação de estruturação genética e a interpretação das possíveis

barreiras ao fluxo gênico dentro e entre as espécies (ex. Eizirik et al., 2001), gerando um aumento do conhecimento sobre os processos históricos biogeográficos.

Entre os marcadores moleculares mais frequentemente empregados em estudos populacionais, evolutivos e/ou voltados para conservação destaca-se o DNA mitocondrial (mtDNA), como marcador de diversidade genética, por possuir características que fazem desse marcador uma excelente ferramenta, tais como ter herança exclusivamente materna, não sofrer recombinação, conter inúmeras cópias por célula, e em algumas regiões, a evolução é extremamente mais rápida comparada ao DNA nuclear (Nedbal e Flynn, 1998).

Co-ocorrência em Porto Rico: espécies-irmãs

A compreensão da composição da dieta e modo de forrageamento de uma espécie ao longo da sua distribuição é um fator importante para compreender sua biologia. Quando essa espécie co-ocorre com sua espécie-irmã, se torna interessante investigar se há sobreposição de microhabitats utilizados para forrageamento, assim como partição de habitat. No entanto, estratégias como não sobreposição de territórios para alimentação são importantes para a manutenção de espécies simpátricas com sobreposição de itens que compõe a dieta.

Enquanto *C. striatus* possui uma ampla distribuição no Atlântico Ocidental, *C. capistratus* é endêmico do Caribe. Por ser o peixe borboleta mais abundante no Caribe, *C. capistratus* possui uma grande quantidade de estudos, incluindo sobre alimentação, quando comparado com *C. striatus*.

Para verificar como essas espécies lidam com a simpatria, incluímos comparações de taxas de mordidas, abundância, composição da dieta (conteúdo estomacal) e condição nutricional (RNA:DNA).

Chaetodon humeralis

Chaetodon humeralis é a espécie da família Chaetodontidae mais comum do Pacífico Oriental. Sua distribuição inclui a porção central e sul do Mar de Cortez, e as ilhas de Cocos, Malpelo e Galápagos (Thomson et al., 2000; Humann e Deloach, 2004). Essa espécie é geralmente encontrada em pares ou pequenas agregações.

Pouco se sabe sobre a biologia dessa espécie, principalmente quanto a composição da dieta e forrageamento. Essa região onde *C. humeralis* ocorre é de certo modo similar ao sudeste-sul do Brasil, com águas frias e recifes rochosos. Portanto, se torna interessante comparar a ecologia alimentar e condição nutricional com *C. striatus*, uma vez que as outras duas espécies do subgênero *Chaetodon* ocorrem em regiões tropicais.

Objetivos

Avaliar a ecologia alimentar de três espécies de peixes-borboleta do subgênero *Chaetodon*, sendo: oito populações de *C. striatus*; uma de *C. capistratus* (Atlântico Ocidental) e; uma de *Chaetodon humeralis* (Pacífico Oriental). Também foi avaliada a variabilidade genética e a conectividade entre as populações de *C. striatus* e influência de aspectos físicos (estrutura física do ambiente e temperatura da água) e biológicos (disponibilidade e tipo de alimentos) sobre a condição nutricional das três espécies.

Objetivos Específicos

- Comparar os tipos de substrato recifal selecionados para alimentação e a influência da temperatura na frequência alimentar das espécies nos diferentes sistemas recifais (rochoso e biogênico);
- Verificar se as espécies apresentam seletividade de microhabitats de forrageio, ou se utilizam de acordo com a disponibilidade;
- Comparar a condição nutricional de *C. striatus* ao longo da costa brasileira e também comparar as três espécies, através da razão RNA-DNA;
- Analisar a variabilidade genética intra e inter-populacional de *Chaetodon striatus*;
- Analisar padrões filogeográficos das espécies ao longo da sua distribuição;

Metodologia utilizada

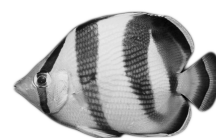
Para desenvolver esse trabalho, foram utilizadas as seguintes metodologias: observação do forrageamento de indivíduos através da técnica “animal focal” (Lehner, 1996); análise da composição do substrato através de fotoquadrados (Figura 3a; Preskitt et al., 2004); análise do conteúdo estomacal de aproximadamente 20 indivíduos de cada localidade amostrada (frequência de ocorrência, volume e Índice alimentar – Iai); análise da condição nutricional através da razão RNA:DNA; análise filogeográfica de *Chaetodon striatus*. Foram incluídas outras duas espécies de peixes borboleta, *Chaetodon capistratus* (Caribe) e *C. humeralis* (Pacífico). As localidades amostradas estão na tabela 1.

Tabela 1. Pontos de coleta, o tipo de ambiente e a localização.

Local de coleta	Tipo de recife	Latitude/longitude
Ilha do Arvoredo, SC	rochoso	27°17'S, 48°22'W
Arraial do Cabo, RJ	rochoso	23°S, 42°W
Abrolhos, BA	coral	17°58'S, 38°42'W
Guarapari, ES	rochoso/com cobertura de coral	20°S, 40°W
Ilha de Trindade Salvador, BA	rochoso com cobertura de coral	20°30'S, 29°20'W 12°S, 38°W
Tamandaré, PE	com cobertura de coral	8°44'S, 35°05'W
Porto Rico, EUA	coral	17°56'N, 67°01'W
Baja California, México	rochoso	23°59'N, 109°49'W



Figura 3: A) Mergulhador realizando fotoquadrado; B) prancheta de PVC para anotações das observações em campo; C) poliquetas encontrados no conteúdo estomacal e; D) procedimentos laboratoriais para análise de razão RNA:DNA.



Artigo I

**Diet, foraging activity and nutritional condition of the banded
butterflyfish *Chaetodon striatus* along the Western Atlantic
Ocean**

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Diet, foraging activity and nutritional condition of the banded butterflyfish *Chaetodon striatus* along the Western Atlantic Ocean

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Abstract

The present study is the first to compare three complementary approaches to understand feeding behavior of a generalist butterflyfish along its distribution range in the Western Atlantic Ocean: foraging mode, stomach contents and nutritional condition. There were no major differences in body size and density across populations. However, differences in feeding rates among seven populations were detected but they did not follow the expectation that fishes that feed on sessile invertebrates have lower abundance and feeding rates out of the tropics. Electivity indices did not show selectivity patterns among sites, suggesting that *Chaetodon striatus* has high foraging plasticity. We analysed 159 stomachs and 81 tissue samples for RNA:DNA ratio. Fifty-three different items were found in the stomach contents, including Annelida, Arthropoda, Echinodermata, Cnidaria and Polychaeta. The number of items ranged from 11 to 31 per locality and Polychaeta was the most frequent item (60% of the Feeding Index). All localities, except Salvador (NE Brazil), had similar RNA:DNA ratios, suggesting that *C. striatus* nutritional condition does not change along its extensive distribution. Despite the large variety of small mobile invertebrates found in *C. striatus* stomach contents, sessile invertebrates comprised the higher Feeding Index (83%). *Chaetodon striatus* should be considered a generalist zoobenthivorous that forages on a wide variety of invertebrates.

Keywords: Chaetodontidae, feeding ecology, selectivity, Ivlev's electivity indices.

Introduction

The feeding ecology of a species is an important aspect of its biology. The relationship between food availability, food quality and physiological state will likely reflect in its distribution and abundance and also will directly influence its feeding behavior and prey types (Gerking 1994).

Butterflyfishes (Chaetodontidae) are known to live highly associated to the substrate and to consume a variety of benthic prey items. Their diet consists mostly of anthozoans, polychaetes and small crustaceans both in the Pacific and in the Caribbean (Randall 1967, Birkeland and Neudecker 1981; Pratchett 2005). Some species feed exclusively on scleractinian corals (obligate corallivores), others feed mainly on corals but supplement their diets with other invertebrates (facultative corallivores) and some species very rarely (or never) feed on corals (generalists). In addition, there are a few species that may also complement their diet feeding on plankton (Sazima and Sazima 2001; Pratchett 2005). Most studies on feeding activity of butterflyfishes are focused on Indo-Pacific coral feeders. Some of these species are wide-ranging mostly because the distribution of coral reefs in the Indo-Pacific is quite significant (Hobson 1974; Irons 1989; Tricas 1989; Pratchett 2005; Gregson et al. 2008).

Observations of fish feeding behaviour can be used to estimate bite rates and as a suggestion of the diet of the species, based on broad food categories (e.g., Birkeland and Neudecker 1981; Lawton et al. 2011). For some species it is possible to identify dietary items directly by observing them in the field. Usually, the identification of food source and inferences of diet composition based on field observations is possible for specialized fish species, such as corallivores (Pratchett 2007). Yet, for generalist species that browse minute preys in different substrates, is impracticable to identify their diet based only on direct observation. Several small invertebrates (i.e. crustaceans, polychaetes and anthozoans) are hidden among algae-dominated benthic microhabitats, such as the epilithic algal matrix (EAM) or sand (e.g., Harmelin-Vivien and Bouchon-Navaro 1983; Kramer et al. 2012), and on the surface of scleractinians and non-scleractinians anthozoans (e.g. Gleibs et al. 1995; Nagelkerken et al. 2009). However, studies evaluating the benthic microhabitats where generalist butterflyfishes browse their preys are scarce. This knowledge provides important information to the

understanding of foraging plasticity of microhabitats, feeding selectivity and the relationship of species distribution range and availability of the microhabitats.

Because of the difficulty in identifying prey items of generalist species in the field, analyses of stomach contents are important to identify and quantify cryptic components that contribute to the diet of these species. Studies on the stomach contents of Chaetodontidae species have been well documented in the Indo-Pacific (e.g., Hiatt and Strasburg 1960; Harmelin-Vivien and Bouchon-Navaro 1983; Sano 1989) and in the Caribbean (e.g., Randall 1967; Birkeland and Neudecker 1981; Pitts 1991). However, few studies investigating possible differences within species in large geographic scales are known. Lawton et al. (2011) found that different populations of some butterflyfish species in the Pacific can exhibit high feeding plasticity (i.e. dietary items and feeding habits).

The banded butterflyfish, *Chaetodon striatus*, has the widest distribution range among Chaetodontidae in the Western Atlantic Ocean, encompassing two different biogeographic provinces, a wide range in sea water temperature (17–30°C; as well as seasonal fluctuations) and different reef formations (i.e. coral and rocky reefs) (Floeter et al. 2008). Its range spans 10,000 km, from Florida, U.S.A. to the south coast of Brazil. All these factors may result in different food availability that should reflect in dietary plasticity. The few studies available investigate diet at local scales (Pitts 1991; Sazima and Sazima 2001; Bonaldo et al. 2005; Dubiaski-Silva et al. 2008). The known diet of *C. striatus* comprises non-scleractinian anthozoans and polychaetes. Therefore there is need for a broad macroecological understanding of its foraging behaviour.

Species abundance and nutritional condition is predicted to be lower near the edges of their geographic range where environmental conditions may become increasingly adverse (Brown 1984; Gaston et al. 1997). However, very few species had been studied throughout their entire range (Sagarin and Gaines 2002), and exceptions to the ‘abundant centre distribution’ hypothesis are appearing in the literature (Jones 2002; Sagarin and Gaines 2002; Tuya 2008; Hobbs 2011). Considering the tropics as the abundant centre for tropical reef fishes, when one moves to higher latitudes it is expected that fishes feed in lower rates because of slower metabolism rates in colder waters (Floeter et al. 2004). It is also likely to live longer and grow to larger sizes (Choat and Robertson 2002).

The wide distribution of *C. striatus* makes it a good candidate for testing feeding plasticity and nutritional ecology in face of natural and anthropogenic environmental gradients. Species nutritional condition influenced by diet type and water temperature can be investigated through RNA:DNA ratio analyses (Buckley and Szmant 2004; Behrens and Lafferty 2007). This index has been broadly employed once that RNA protein synthesis varies with metabolic demand, i.e. fluctuates in response to food availability (quality) while DNA content per cell is fixed (Calderone et al. 2001; Chícharo and Chícharo 2008).

We conducted the first geographic-extensive investigation of a non-obligate coral-feeding species (*C. striatus*), from the Caribbean to south Brazil. The present study aims to fill a gap in the knowledge of the nutritional ecology of *C. striatus*. The specific goals of this study were to evaluate: 1) if there are differences in density, body size and feeding rates of *C. striatus* along its distribution range; 2) if the substrate where they forage either reflects what is available on the benthic coverage or they target specific microhabitats; 3) the dietary composition of *C. striatus* along its distribution range and; 4) the nutritional status (fitness) through RNA:DNA ratio in different *C. striatus* populations.

Methods

Study area

Fieldwork was carried out in two types of reef systems: coral reefs (Puerto Rico; Tamandaré; Salvador and Abrolhos) and rocky reefs (Trindade Island; Guarapari; Arraial do Cabo and Florianópolis) (Fig. 1; Table 1). This area encloses almost all of *C. striatus* distribution range, being the southernmost limit included, once that towards south from Florianópolis there is a gap on rocky reefs of almost 600 km.

Water temperature was measured during each dive and the mean water temperature recorded among sites is within the minimum and maximum temperature range documented for each location (Table 1; Ferreira et al. 2004; Prada et al. 2010; Selig et al. 2010; NOAA - <http://www.nodc.noaa.gov/sog/cortad/>). For the analyses, we used NOAA minimum average water temperature in the last ten years.

Foraging behavior

Fieldwork was carried out between January 2010 and March 2012 using SCUBA diving in all sites except Trindade Island. Underwater observations were conducted during daytime, between 9:00 and 16:00, summing over 140 hours of sampling effort in depths ranging mostly between 2–8 m, except Puerto Rico where the reefs had 20 m depth.

Foraging behavior was quantified by following individual fishes (focal animal methodology; Lehner 1996) for 3-min periods and counting the number of bites taken from each category of the benthic coverage (see details below). Individuals were selected haphazardly, with a minimal of 57 fish observations per locality. To minimize the risk of resampling the same individual, the observers moved through the sampling site to search for other individuals (Birkeland and Neudecker 1981). We waited for a few minutes before we started the counting, in order to allow the fish to get used to the diver. Fish were then followed at a discreet distance (1–3 m) where fish did not appear to be affected by the observer (Birkeland and Neudecker 1981; Alwany 2003). We sampled only adult individuals (>10 cm), to ensure that results were not biased by potential ontogenetic changes in feeding.

The photoquadrat method (Preskitt et al. 2004) was used to estimate the relative abundance of each substratum type. Five random photos (covering an area of 40 x 60 cm; Krajewski and Floeter 2011) were taken in an area of approximately 2 x 2.5 m (5m²) around the last bite taken by the fish being sampled (i.e. the end of each 3-min observation).

Fish density

In order to evaluate the abundance of *Chaetodon striatus* along its range, a minimum of 100 belt transects (20 x 2 m; details in Floeter et al. 2007) was conducted at each sampling locality, except at Puerto Rico where we obtained published online data (La Parguera; data from: http://www8.nos.noaa.gov/bioge_public/query_fish.aspx - collected by belt transects of 100 m²).

Diet

Fishes were collected using hand spears and stomach content was immediately stored after collection in tubes with ethanol. We collected 17 up to 30 fishes at each sampled site. Food items were identified under a stereomicroscope to the lowest suitable taxonomic category and listed. The volume of each item was measured in a 1mm high square-shaped transparent dish with a scale in millimetres underneath so that the area corresponded to the volume. The relative importance of each item was analysed through the Feeding Index (IA_i), which combines frequency of occurrence and volume of each item, in relation to the total (Kawakami and Vazzoler 1980). When we found masses of organic material that seemed to be unidentifiable, they were analyzed under microscope in search for clue elements. Contents were classified as “*digested organic matter*” when elements such as spicules, nematocysts, setae were found mixed within the organic matter. When we observed only nematocysts, in high abundance among the organic matter we classified them into Cnidaria Hexacorallia Actiniaria or Cnidaria Hexacorallia Zoantharia categories. However, it was not possible to distinguish the nematocysts from Cnidaria Hexacorallia Corallimorpharia and Scleractinia (Mariscal 1974), so we referred to them as Cnidaria Hexacorallia “Corallim/Scle”. When there were mixed nematocysts we classified as Cnidaria Hexacorallia “other”. Also, when no identifiable elements were found, we classified as “unidentifiable”. Several taxonomic experts were consulted to confirm or provide identification of dubious items. Data was arranged in 21 groups, as shown in the results.

Total length (TL) of each fish and full non-stretched stomach length from the pyloric duct to the rectum was measured.

Nutritional condition

RNA:DNA ratio is a physiological index that is sensitive to changes in dietary status of organisms (Buckley and Szmant 2004; Behrens and Lafferty 2007). In order to determine RNA:DNA ratios, white muscle of approximately 20 fishes from four populations (Puerto Rico, Salvador, Guarapari and Florianópolis) was stored in RNALater (Quiagen) solution immediately after sampling and kept in -20°C freezer. Samples were thawed and we first determined RNA and DNA concentrations of each sample using ethidium bromide fluorescence (Bentle et al. 1981, modified by Dahlhoff and Menge 1996). We weighted samples to 100 mg and homogenized them in replicates

with 10 volumes of 2 mol NaCl. The samples were centrifuged for 1 min, and then 5 μ l of each sample was transferred into a microplate adding 0.14 μ l of solution containing 0.005 mg ethidium bromide and 0.15 mg proteinase K at 37°C for 60 min. After this initial incubation, we added 100 μ l of buffer (80mm Tris-HCl, pH 8.0 at 20°C) and then measured fluorescence at 365 nm excitation and 590 nm emission using a SpectraMax M5 spectrofluorometer (Molecular Devices). We determined fluorescence due to RNA and DNA by sequential digestion of each nucleic acid using first 5 μ l RNase I (SIGMA) followed by 60 min incubation and then 5 μ l DNase I (SIGMA) followed by 30 min incubation. Last, we estimated RNA and DNA concentrations from a standard curve calculated by measuring the fluorescence of known quantities of RNA and DNA.

Data analysis

The benthic cover in each photoquadrat was classified into 11 microhabitat categories: six algae-dominated types (epilithic algal matrix [EAM], crustose, foliose, leathery, corticated, articulated calcareous), Anthozoa (Octocorallia, Zoantharia, and Scleractinia), and other microhabitats (e.g. porifera and sand). To analyze the photographs we used the software Coral Point Count with Excel Extension (CPCe v3.5 - Kohler and Gill 2006), where 20 random points was added on each photograph to estimate the relative cover of each microhabitat.

Ivlev's Electivity Index ("E" in Krebs 1989) was used to evaluate feeding selectivity, comparing what microhabitats items are used for feeding with respect to their availability. The E values ranges from -1 to 1, in which values close to 1 indicate preferences and values close to -1 indicate avoidance (Krebs 1989). We tested for preference/avoidance with a null model that generated expected values for "E", taking into account the number of bites and the availability of each benthic item. The total number of bites per individual was randomized across benthic categories (1,000 randomizations) and then employed to obtain a null distribution of E in the absence of selectivity. Confidence limits were determined using the percentile method (Manly 1997) and preference or avoidance was considered statistically significant if the observed "E" was higher or lower than 95% of the randomized datasets. The null model was generated using the R software (R Development Core Team 2012).

One-way ANOVA was used to compare the density and feeding rates among all localities. When significant, *post-hoc* tests (SNK) were carried out to investigate such differences. Regression analysis was conducted to determine the influence of thermal gradient on density, mean bite rate and body size. Data met the assumptions for normality of residuals and homogeneity of variances (Zar 1999; Underwood 1997).

Results

Mean bite rates were statistically different among localities (ANOVA $p < 0.001$), however, no clear geographical gradient was found. Mean abundance of *Chaetodon striatus* was similar along its latitudinal distribution, and not statistically different among reef systems (Fig. 2).

The benthic community differed among localities but with neither a latitudinal nor a temperature-driven pattern. However, a decline in anthozoans towards the southernmost sampling site was observed (Fig. 3). The most available microhabitats were: EAM, at five localities (Puerto Rico 36%, Abrolhos 44%, Guarapari 32%, Arraial do Cabo 24% and Florianópolis 42%); Anthozoa Zoantharia at Salvador (49%); and sand cover at Tamandaré (36%; Fig. 3).

The percentage of bites on each microhabitat was also different among sites. At Puerto Rico, the highest bite rate was on EAM (44.2%); at Tamandaré on foliose algae (37%); at Salvador on Anthozoa Zoantharia (62.3%); at Abrolhos on Anthozoa Scleractinia (42%); at Guarapari and at Arraial do Cabo on Anthozoa Octocorallia (54.4 and 31.3%, respectively) and at Florianópolis bites were mostly on crustose algae (mainly *Codium* spp.; 40%; Fig. 3).

There was no obvious pattern of selectivity/rejection for *C. striatus* along its distribution range, with different results at each site, mostly within the expected interval generated by the null model (Fig. 3). Despite the fact that EAM appeared as a major benthic cover in five sites, it was only selected by *C. striatus* in Puerto Rico (Fig. 3). In Tamandaré we observed that fishes positively selected articulated calcareous algae while crustose algae were rejected. In Salvador we observed rejection on crustose and foliose algae, which were also rejected at Guarapari together with articulated calcareous algae and sand. Fishes in Arraial do Cabo rejected Porifera. In Abrolhos and

Florianópolis all microhabitats were used for feeding at the expected proportions (Fig. 3).

A total of 159 individuals of *C. striatus* were collected. The main quantitative results of stomach contents are presented in Table 2. The majority of *C. striatus* diet was composed by Cnidaria and Polychaeta. *Chaetodon striatus* stomach contents encompassed 53 different items, mainly comprised by Polychaeta, Arthropoda and Cnidaria. The number of items found in each location ranged from 11 to 30 (Salvador and Puerto Rico, respectively).

Mollusca and/or Crustacean eggs were found in all localities in different proportions (Table 2). We excluded eggs from Fig. 1 since they are consumed opportunistically and may bias the understanding of the 'regular' diet. The following description is based on proportions excluding eggs.

In Puerto Rico, 56% of Feeding Index from the items in the stomach contents were Polychaeta (Fig. 1), present in all but one individual stomach. Half of the stomachs analysed presented Cnidaria Hexacorallia. In Tamandaré, Polychaeta represented 84% of the Feeding Index. In Salvador, 95% of the Feeding Index was attributed to Cnidaria Hexacorallia, of which 36.5% was Zoantharia. In Abrolhos, 54% of stomach contents was “Corallim/Scle”, 23% was Polychaeta and 15.3% was other Hexacorallia. In this locality, from 20 stomachs analysed, only two did not have cnidarians. At Trindade Island 63.5% of stomach contents were Polychaeta and 35.5% Cnidaria Hexacorallia. This was the only site in the Brazilian coast where we found the item Octocorallia (20% frequency of occurrence). In Guarapari, 82% of stomach contents was on Cnidaria Hexacorallia, from which 60% was represented by Zoantharia. Despite Arraial do Cabo presented the most variable number of items in the stomach contents on the Brazilian coast, the Feeding Index showed that the bulk of stomach contents was composed on 81.5% of Polychaeta and 15.8% Cnidaria Hexacorallia. At the southernmost limit of distribution of *Chaetodon striatus*, Florianópolis, 85.6% of the Feeding Index was composed by Cnidaria Hexacorallia, from which 34.4% was represented by “Corallim/Scle”, and 29.2% of Actiniaria (Fig. 1).

When comparing the mean total length of fishes of each sampling site, we checked that there was no evidence of changes in body size along *C. striatus* distribution range (Fig. 1). However, the largest fish observed was in Florianópolis (17 cm) and was larger

than described in the literature (15 cm, Kuitert 2002; 15.2 cm Human and Deloach 2002; 15 cm Carvalho-Filho 1999). Linear regression analyses were non-significant when comparing the minimum average water temperature for the last ten years (NOAA-<http://www.nodc.noaa.gov/sog/cortad/>) with density ($n = 7$; $F = 2.5$; $d.f. = 5$; $r^2 = 0.20$; $p = 0.17$), mean body size ($n = 7$; $F = 0.36$; $d.f. = 5$; $r^2 = 0.6$; $p = 0.57$) and mean bite rates ($n = 7$; $F = 0.43$; $d.f. = 5$; $r^2 = 0.08$; $p = 0.84$).

Chaetodon striatus individuals measured between 12.8—14.5 cm and the stomach length was between 37.5—106 cm. The relative stomach length resulted in a range between 2.9—7.3 and the mean was 4.5 (± 0.26 SE) (Fig. 4). Results of RNA:DNA ratio measured in samples from Puerto Rico, Salvador, Guarapari and Florianópolis showed that almost all populations have similar values except fishes from Salvador (Fig. 5), which presented a significantly lower ratio (1.55 ± 0.69 SD).

Discussion

This study presents a complete evaluation on feeding and nutritional condition of a generalist butterflyfish species along its distribution range. Foraging behavior and stomach content analysis are complementary approaches crucial to understand the feeding habits of generalist butterflyfishes. While the first method is based in field observations, the second identifies cryptic dietary species that were not possible to detect while observing fish feeding (Nagelkerken et al. 2009; Kramer et al. 2012). Finally, the nutritional condition reveals the physiological status of the species along its distribution range.

Chaetodon striatus did not present a pattern of foraging microhabitat selectivity, foraging in accordance to what was available, even though the benthic composition of reefs varies significantly along the Western Atlantic. When comparing the observed results of feeding selectivity against the expected random interval, we observed that very few microhabitats overcame expected values. This absence of selectivity/rejection suggest that differences in composition of the benthic coverage along almost 44° of latitude (from the Caribbean to South Brazil) is not an obstacle for *C. striatus* establish new populations. These results point out that this species is a browser of small invertebrates that inhabit varied microhabitats (e.g. sand and algal dominated substrata).

Chaetodon striatus feeds on a large variety of taxonomic groups, varying its dietary composition along its geographic gradient. However, polychaetes and cnidarians were the most common groups eaten in all populations.

Previous studies in the Caribbean with *C. striatus* stomach contents also found high volumes of polychaetes and anthozoans (58.7% and 32.5% respectively; Randall 1967). In the case of *C. striatus*, because of its large distribution range, we would expect that in sub-optimal localities, as in the marginal rocky reefs, the species tends to choose items with higher energetic values such as mobile invertebrates. However, polychaetes and anthozoans were the most important dietary items along *C. striatus* distribution range. In all populations, at least 50% of Feeding Index was composed by one of these groups.

Polychaetes are an important group of the benthic community of reef systems that associates with different microhabitats, such as algae, sand, and corals. This explains why *C. striatus* forages most in microhabitats like algal patches and sand. The most important family in *C. striatus* diet was the tube dwellers Sabellidae. We found either entire polychaetes or only tentacular crowns. This could be in response to the fact that polychaetes shrink fast enough so the Chaetodontidae can only ingest the tentacular crowns.

Anthozoans were also an important dietary item, representing high Feeding Index in all localities, except Tamandaré. The result of stomach contents is corroborated by field observations, where high proportions of bites in at least one of the categories of anthozoans were observed in almost all localities. However, two localities had high bite rate on octocorals, Arraial do Cabo (mostly the sea fan *Phyllogorgia dilatata*) and Guarapari (mostly *Plexaurella* spp.) but we did not observe this item in the stomach contents of the species. We believe that the small polyps are easily digested therefore they were not detected by our analysis. Furthermore, we searched for the presence of sclerites in a attempt to detect consumption of octocorals, but *Phyllogorgia dilatata* and *Plexaurella* spp. polyps sclerites are weak or absent (Castro et al. 2010; Medeiros, pers com) and, therefore, can be easily degraded by the fixation process and digestion. From all *C. striatus* individuals in the present study, Cnidaria Octocorallia was present only in Puerto Rico and Trindade Island, both with IAI <0.5%.

Conversely, stomach contents of fishes from Florianópolis (south Brazil) had high presence of anthozoans (75% IAI) while field observations did not recorded bites on this

item. Because most of the bites on this locality were on algae dominated substrate, it is likely that fishes were searching for cryptic species, not easily noticeable during field observations.

Both cases exemplify the importance of combining data from field observations with stomach content analyses to understand the feeding behaviour of a species, once each approach alone may present contrasting results. Additionally, we recorded scleractinian corals in *C. striatus* diet for the first time.

This study revealed a high diversity of dietary items, some with high frequency of occurrence and low volume, reflecting in low value of Feeding Index. Some of these items are probably an important complementary resource, due to their high energetic value (Nagelkerken et al. 2011). An example is the presence of crustaceans in all sampled sites, except Salvador, in which the Feeding Index was $<0.5\%$ (Table 2). The presence of items such as algae, sand and calcareous skeleton most probably indicates incidental ingestion while targeting small invertebrates.

Another item that provides high energy is egg mass (Fyhn and Govoni 1995), which was found in five sites. We highlight the results of Tamandaré, where we found high volume of eggs mass, corresponding to 3/4 of all stomach contents. Since this is a sporadic item, we suggest that this is an opportunistic behaviour. When eggs masses are excluded of the results, polychaetes turn to be the most important dietary component.

Previous study described that *C. striatus* has increased relative abundance towards subtropical rocky reefs (Ferreira et al. 2004). In this study, *C. striatus* abundance was not variable along its geographical gradient, a result that is similar for generalist species of the Indo-Pacific (Pratchett et al. 2006).

Feeding rates varied among populations in this study. However, both populations in the extremes of sampling did not differ statistically, contrary to the expected hypothesis. In previous studies with *C. striatus*, Bonaldo et al. (2005) found for one population at southeastern Brazil a mean bite rate range of 1.5–2.3 bites/min and Osorio (2006) found for a population at Salvador a mean bite rate of 2.3 (± 1.36 SD). Comparing to our results, Puerto Rico's population had a slightly lower bite rate (1.38 bites/min.) and Guarapari population had the highest mean bite frequency (3.5 ± 0.29 SE). Considering all subtropical rocky reefs, the mean bite rate of *C. striatus* (2.5 ± 0.8 SE) was within

the previously described range (Bonaldo et al. 2005; Osorio 2006). Again, our results did not support the hypothesis that *C. striatus* has lower bite rates in higher latitudes.

Other obligate and facultative coral-feeding species have their feeding rates 2–6 times higher than *C. striatus* (Irons 1989; Alwany et al. 2007; Gregson et al. 2008), most likely in order to obtain their energetic needs from a low caloric diet (Floeter et al. 2004). Conversely, *C. striatus* is a generalist, with high caloric items in its diet, such as polychaetes and crustaceans, and this might explain the lower comparative feeding rates detected in this species.

Gut morphology can vary among conspecifics (Raubenheimer and Bassil 2007). This explains why we found fishes with same TL, but stomach length varying in 30 cm (39.5 and 69.5 cm). Berumen et al. (2011) compared the relative stomach length of 28 species of butterflyfishes that are obligate coral-feeders, facultative coral-feeders, generalists, non coral-feeders and planktivores. Comparing our results of relative stomach length, *C. striatus* fits in the range of the subset, which includes generalists and non-coral feeders (between 4 and 5 mm/mm of relative stomach length – Fig. 4).

The analysis of RNA:DNA ratio indicated that all populations sampled had similar nutritional condition. Even though we did not have samples from all populations, we were able to include individuals from the extremes of our sampled sites (Puerto Rico and Florianópolis), which include at least one extreme of distribution in subtropical rocky reefs with environmental conditions that can be unfavourable for tropical coral-reef fishes. Despite the fact that fishes from Salvador showed statistically low RNA:DNA ratio, this value was not lower than 1, the boundary value considered as an indicative of physiological stress, such as starvation (Kono et al. 2003; Beherens and Lafferty, 2007). The result from Salvador population may be in accordance to the fact that 95% of the stomach contents were Cnidarians. At the field, it was observed a higher cover (49.7%) and a high bite rate (62.3%) on Hexacorallia Zoantharia microhabitat. Cnidarians are known to provide low energetic quality and nutritional value because of the presence of chemical (e.g., palytoxin) and structural defences (e.g., carbonate skeletons; Birkeland and Neudecker 1981; Suchanek and Green, 1981; Fox 1982).

Although *C. striatus* was previously categorized as “sessile invertebrate feeder” (Ferreira et al. 2004), our results point out that its diet is more versatile than previously reported. Even though quantitatively most of the items present in *C. striatus* stomach

content were mobile invertebrates, it represented 17% of IAI (37% FO e 18% V), while 83% of total Feeding Index (62% FO e 81% V) was composed by sessile invertebrates. However, the presence of low quantities of mobile invertebrates is highly important in terms of nutritional value. The high dietary plasticity exhibited by *Chaetodon striatus*, ingesting a vast variety of benthic items lead us to consider this species a generalist zoobenthivore. Furthermore, its strategy seems to be successful in its wide variety of reef environments, temperature ranges, and resource availability, since its nutritional condition is maintained along its geographical range (including southern extreme).

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Legends

Table 1. *Chaetodon striatus* Mean body size, temperature range with the minimum and maximum mean temperature in the last ten years (NOAA), mean temperature at dive, geographic coordinates, coral richness (number of Scleractinia and Octocorallia) and reef type at all sampling sites.

Table 2. Frequency of occurrence (%FO), Volume (%V) in the diet and Feeding Index (%IAi) of all dietary items found for *Chaetodon striatus* populations in eight study sites along the Western Atlantic.

Figure 1. Map of sampling sites along *Chaetodon striatus* distribution range (gray line). Pie charts represent the Feeding Index based on stomach analyses. Egg masses were excluded from these calculations as it is an occasional food item (data available in Table 2).

Figure 2. A) mean bite rates, and B) density of *C. striatus* at each studied site. Letters indicate statistical grouping differences Newman-Keuls SNK *post hoc* test.

Figure 3. Percentage of benthic cover (white bars) and bites (black bars) at each sampling site. Algae: turf; MCRO: crustose, MFOL: foliose, MCOR: leathery, MCT: corticated, MCA: articulated calcareous; Anthozoans: ANT OCT Octocorallia, ANTZOA Zoantharia, ANT SCL Scleractinia; POR: Porifera and Sand. * statistically significant.

Figure 4. Relative gut length of *C. striatus* compared to other butterflyfishes. The information displayed by the white bars was extracted from Berumen et al. (2011).

Figure 5. RNA:DNA ratio of *Chaetodon striatus* on four different studied populations along the Western Atlantic. Sample sizes are: Puerto Rico (20), Salvador (19), Guarapari (18) and Florianópolis (20). * statistically significant.

Table 1. *Chaetodon striatus* Mean body size, temperature range with the minimum and maximum mean temperature in the last ten years (NOAA), mean temperature at dive, geographic coordinates , coral richness (number of Scleractinia and Octocorallia) and reef type at all sampling sites.

	Mean body size (cm)	Temperature Range (°C)	Mean temperature at dive (°C)	Geographic coordinates	Corals richness Scleractinia/ Octocorallia	Reef type
Puerto Rico	13.7 ± 0.8	26–30.3	27	17°56'N	117/99	Coral reef patches; mainly dominated by sea whip
Tamandaré	8.5 ± 1.5	25.6–30.7	28	67°01'W 8°44'S	14/5	Beach rock, mainly dominated by urchin barrens and macroalgae
Salvador	8.7 ± 1.7	24.7–30.9	28	35°05'W 12°S	17/6	Rocky reef; mainly dominated by zoanthids
Abrolhos	10.7 ± 2	23.8–29.9	27.5	38°W 17°58'S	15/13	Coral reef; large reef complex; fringing reef; mainly dominated by scleractinians
Trindade	13.1 ± 0.2	22.8–28.4	-	38°42'W 20°30'S	11/-	Volcanic rocky reef; isolated oceanic island; mainly dominated by coralline algae
Guarapari	13 ± 1.9	22–29	23	20°S	14/13	Rocky reef; mainly dominated by octocorals
Arraial do Cabo	10.7 ± 2	20.9–27.1	22	40°W 23°S	5/11	Rocky reef; mainly dominated by sea fan
Florianópolis	12 ± 1.9	17.3–27.7	18	42°W 27°17'S	1/4	Rocky reef; dominated by macroalgae
				48°22'W		

Table 2. Frequency of occurrence (%FO), Volume (%V) in the diet and Feeding Index (%IAi) of dietary items of *Chaetodon striatus* from populations in the eight study sites along the Western Atlantic. [data including eggs.]

Locality		Puerto Rico			Tamandaré			Salvador			Abrolhos		
n° of stomachs		30			17			21			20		
n° of items		30			15			11			20		
		%FO	%V	%IAi	%FO	%V	%IAi	%FO	%V	%IAi	%FO	%V	%IAi
Algae		23.3	0.5	0.2	5.9	<0.1	<0.1	4.8	0.2	<0.1	40.0	1.5	1.7
Cnidaria		10.0	0.4	0.1	17.6	<0.1	<0.1	4.8	<0.1	<0.1			
Hydrozoa													
Cnidaria -	Octocorallia	6.7	0.1	<0.1									
Anthozoa	Hexac. Actiniaria							9.5	5.5	1.6	15.0	10.0	4.3
	Hexac. Zoantharia	3.3	1.0	0.1				33.3	35.9	36.5			
	Hexac. Corallim./ Scle.	60.0	31.8	32.4	29.4	5.6	2.3	9.5	11.2	3.3	45.0	41.7	54.1
	Hexac. Other	33.3	17.6	9.9	17.6	2.9	0.7	42.9	40.8	53.3	25.0	21.2	15.3
Annelida -	Sabellidae	76.7	20.4	26.6	47.1	2.7	1.8				55.0	11.4	18.0
Polychaeta	Serpulidae	56.7	6.9	6.6									
	Cirratulidae	6.7	1.2	0.1	94.1	14.0	18.3				30.0	3.7	3.2
	Eunicidae	6.7	0.5	0.1									
	Syllidae	6.7	0.1	<0.1							5.0	<0.1	<0.1
	Terebellidae	3.3	0.2	<0.1							25.0	0.6	0.5
	Other	93.3	16.4	22.8				14.3	0.9	0.4	25.0	3.8	1.3
Arthropoda	Amphipoda	10.0	<0.1	<0.1	5.9	<0.1	<0.1				10.0	0.1	<0.1
- Crustacea	Isopoda	3.3	0.1	<0.1									
	Decapoda	6.7	0.1	<0.1							5.0	<0.1	<0.1
	Other	10.0	<0.1	<0.1	17.6	<0.1	<0.1	4.8	0.3	<0.1	10.0	0.1	<0.1
Eggs	Mollusca	33.3	1.3	0.4	94.1	72.3	76.2	9.5	2.6	0.8	15.0	0.1	<0.1
	Crustacea	6.7	0.1	<0.1									
	Other												
Others*		23.3	0.1	<0.1	17.6	0.1	<0.1	14.3	0.1	<0.1	5.0	<0.1	<0.1
Inorganic elements**		60.0	0.5	0.2				57.1	2.3	4.1	5.0	0.2	<0.1
Digested Organic Matter***					5.9	1.3	0.1				5.0	4.5	0.6
Unidentified		36.7	0.8	0.5	35.3	1.0	0.5				25.0	1.1	0.8

Table 2. continued

Locality		Trindade Island			Guarapari			Arraial do Cabo			Florianópolis		
n° of stomachs		10			22			19			20		
n° of items		10			22			27			16		
		%FO	%V	%IAi	%FO	%V	%IAi	%FO	%V	%IAi	%FO	%V	%IAi
Algae		20.0	0.6	0.2	9.1	<0.1	<0.1	26.3	0.3	0.2	40.0	0.2	0.2
Cnidaria	Hydrozoa	50.0	0.2	0.2	36.4	0.3	0.2	15.8	<0.1	<0.1	60.0	0.6	1.0
Cnidaria -	Octocorallia	20.0	0.7	0.2									
Anthozoa	Hexac. Actiniaria	20.0	6.9	2.3							35.0	29.5	28.8
	Hexac. Zoantharia				77.3	48.6	59.4	10.5	7.2	1.5			
	Hexac. Corallim./ Scle.	30.0	6.5	3.2	50.0	10.6	8.4	5.3	0.7	0.1	35.0	34.6	33.8
	Hexac. Other	50.0	28.1	23.4	40.9	20.5	13.3	31.6	20.4	12.9	25.0	17.1	11.9
Annelida -	Sabellidae	70.0	14.5	17.0	45.5	2.8	2.0	89.5	35.9	64.1	15.0	1.2	0.5
Polychaeta	Serpulidae	20.0	2.6	0.9	4.5	<0.1	<0.1						
	Cirratulidae							63.2	8.1	10.2	5.0	0.4	0.1
	Eunicidae	10.0	0.1	<0.1									
	Syllidae				13.6	0.1	<0.1						
	Terebellidae				9.1	1.0	0.1						
	Other	100.0	20.3	33.8	31.8	1.7	0.7	10.5	0.6	0.1	85.0	5.1	11.4
Arthropoda -	Amphipoda	20.0	<0.1	<0.1	45.5	0.3	0.1	73.7	0.5	0.4	10.0	0.1	<0.1
Crustacea	Isopoda							15.8	0.1	<0.1			
	Decapoda	20.0	0.2	0.1				21.1	0.7	0.1			
	Other	10.0	0.4	0.1	18.2	<0.1	<0.1	10.5	0.1	<0.1	10.0	<0.1	<0.1
Eggs	Mollusca	70.0	18.6	18.5	63.6	1.6	1.6	42.1	21.0	8.8	45.0	10.3	11.5
	Crustacea												
	Other				18.2	0.5	0.2						
Others*					40.9	2.1	0.6	57.9	2.0	1.0	5.0	0.1	<0.1
Inorganic elements**		50.0	0.2	0.1	63.6	0.9	0.8	10.5	<0.1	<0.1	5.0	<0.1	<0.1
Digested Organic Matter***								5.3	1.6	0.2			
Unidentified		10.0	<0.1	<0.1	90.9	8.8	12.7	31.6	0.7	0.4	35.0	0.8	0.8

*Composed by Foraminiferida, Ectoprocta, Porifera, Platyhelminthes, Nematomorpha, Echinodermata (Ophiuroidea and Holothuroidea) and Angiosperma.

**Composed by fragments of silica and calcareous skeletons.

***Composed by nematocysts, spicules and setae.

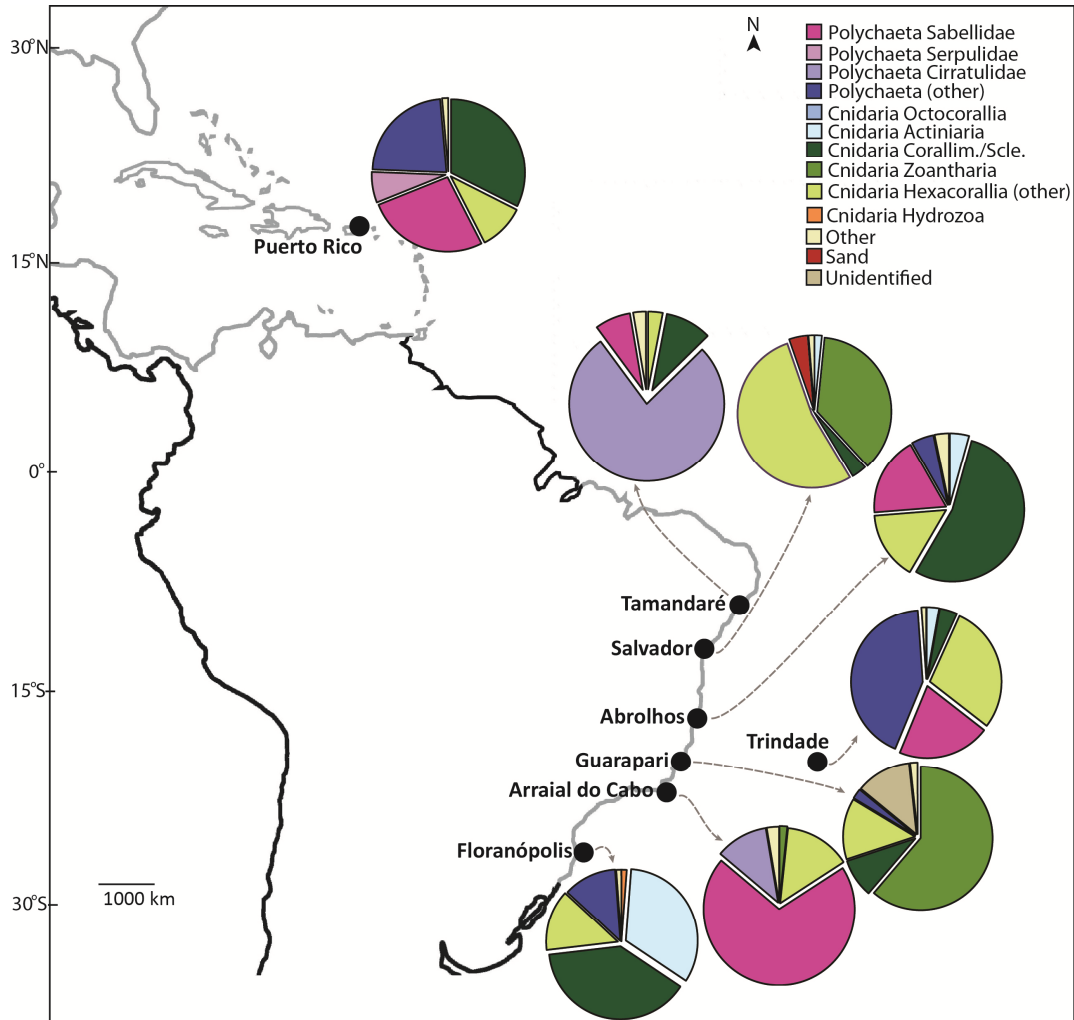


Figure 1. Map of sampling sites along *Chaetodon striatus* distribution range (gray line). Pie charts represent the Feeding Index based on stomach analyses. Egg masses were excluded from these calculations as it is an occasional food item (data available in Table 2).

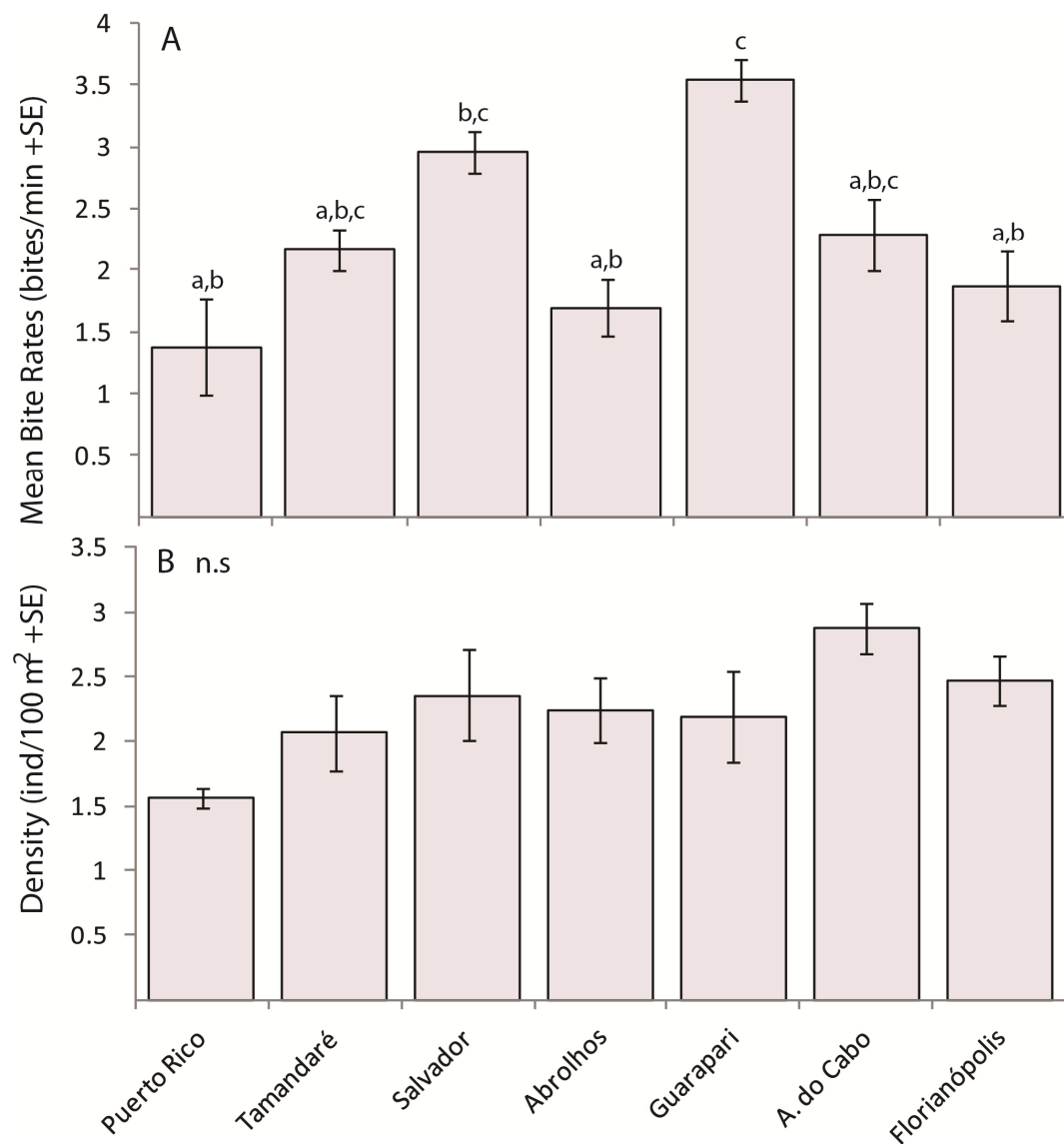


Figure 2. A) Mean bite rates, and B) density of *C. striatus* at each studied site. Letters indicate statistical grouping differences with Newman-Keuls SNK *post hoc* test.

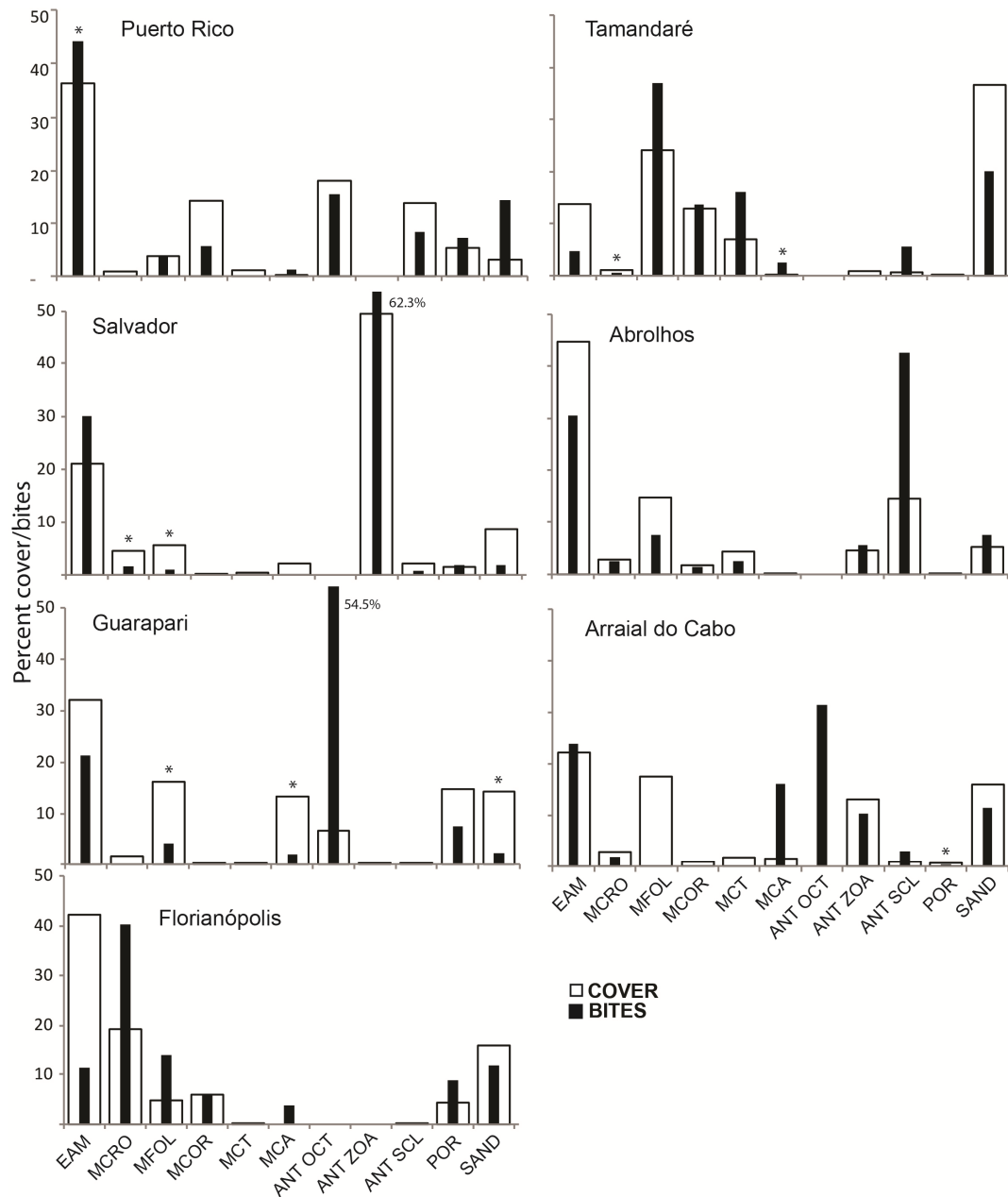


Figure 3. Percentage of benthic cover (white bars) and bites (black bars) at each sampling site. Algae: EAM; MCRO: crustose, MFOL: foliose, MCOR: leathery, MCT: corticated, MCA: articulated calcareous; Anthozoans: ANT OCT: Octocorallia, ANT ZOA: Zoantharia, ANT SCL: Scleractinia; POR: Porifera and Sand. * differences using IVLEV Electivity index and confident limits (randomized).

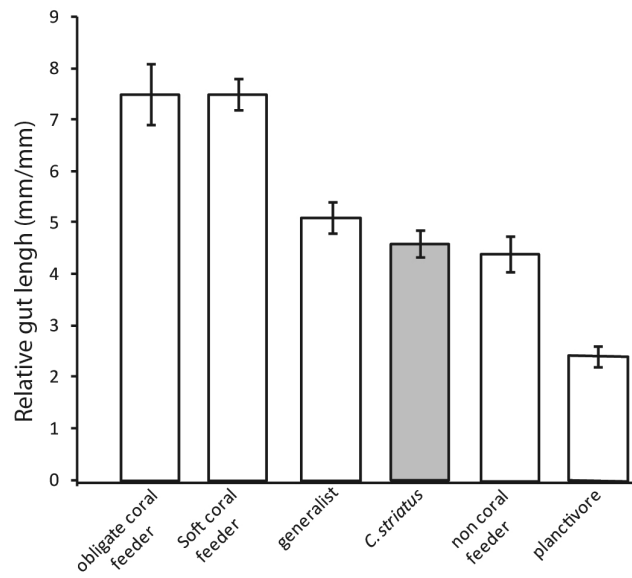


Figure 4. Relative gut length of *C. striatus* compared to other butterflyfishes. The information displayed by the white bars was extracted from Berumen et al. (2011).

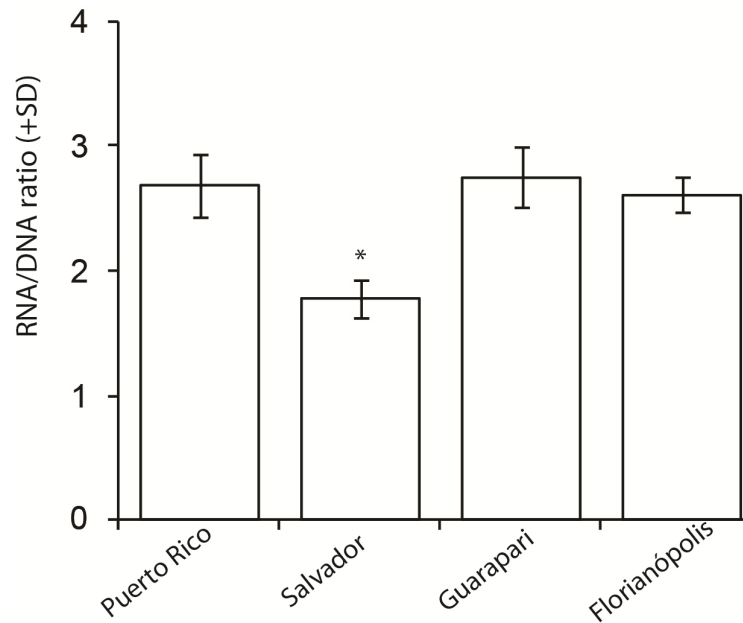
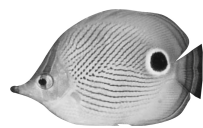


Figure 5. RNA:DNA ratio of *Chaetodon striatus* on four different studied populations along the Western Atlantic. Sample sizes are: Puerto Rico (20), Salvador (19), Guarapari (18) and Florianópolis (20). * indicate statistical differences with Newman-Keuls SNK *post hoc* test.

Artigo II



**Feeding ecology of the sympatric sister-species of
butterflyfishes *Chaetodon capistratus* and *C. striatus* at La
Parguera, Puerto Rico**

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Feeding ecology of the sympatric sister-species of butterflyfishes *Chaetodon capistratus* and *C. striatus* at La Parguera, Puerto Rico

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ABSTRACT

Foraging activity, stomach contents and nutritional condition of the sister-species Chaetodon capistratus Linnaeus, 1758 and Chaetodon striatus Linnaeus, 1758 were studied in La Parguera, Puerto Rico. Underwater observations were used to evaluate foraging selectivity for microhabitats, stomach contents were analyzed for diet, and tissue samples processed to estimate nutritional condition through RNA:DNA ratio. Differences on feeding rates and abundance between species were detected. Chaetodon capistratus took more bites on octocorals and hard corals but did not present selectivity for these items. On the other hand, it rejected low complexity microhabitats close to the reef-sand interface. Chaetodon striatus took more bites on the epilithic algal matrix and sand microhabitats and was usually found close to the reef-sand interface, while C. capistratus was mostly found in the centre of reef patches. This segregation pattern suggests some level of habitat partitioning. Polychaetes and cnidarians were the most representative items in the stomach contents of both species. However, 74.5% of the Feeding Index of C. capistratus was on cnidarians while C. striatus had 56% of the Feeding Index on polychaetes. Both species had similar RNA:DNA ratios, suggesting that the different proportion of items ingested and feeding rates does not have a large influence on their nutritional fitness. Our results support the idea that both species are generalist zoobenthivores, and have adaptations to include mechanically or chemically defended cnidarians in their diets.

Keywords: chaetodontidae, habitat partition, stomach contents, foraging behavior, butterflyfishes, nutritional condition.

INTRODUCTION

The feeding ecology of a species is an important aspect of its biology. The relationship between food availability, food quality and physiological state will likely reflect in its distribution and abundance and also will directly influence its feeding behavior and prey types (Gerking, 1994). An increasing number of studies have reported resource use among sympatric species, including habitat partition in small reef fishes (e.g. blennies – Clarke, 1994; gobies – Munday, 2001).

Butterflyfishes (Chaetodontidae) feeding behaviour have been widely studied (e.g. Pitts, 1991; Zekeria *et al.*, 2002; Pratchett, 2005; Nagelkerken *et al.*, 2009), however no study to date focused in comparing sister-species' foraging behaviour and diet at small spatial scales (e.g. same reef). Explore inter-specific foraging behaviour and diet of sympatric species may lead to a better understanding of species co-existence. The co-occurrence of sister-species may lead to competition for food resources or habitat partition (Anderson *et al.*, 1981; Pitts, 1991; Pratchett, 2005; Bellwood *et al.*, 2006). However, a strategy for sympatric species with dietary overlap may include the maintenance of non-overlapping feeding territories (Wrathall *et al.*, 1992). For example, in general, sympatric corallivore butterflyfishes may have 30–50% of dietary overlap (Pratchett, 2005), but values can be as high as > 70% in assemblages of Hawaii (Cox, 1994).

The subgenus *Chaetodon* (*sensu* Fessler & Westneat, 2007; Bellwood *et al.*, 2010) is formed by two species pairs: *Chaetodon capistratus* and *C. striatus* and its sister clade *C. ocellatus* and *C. humeralis* (Bellwood *et al.*, 2010). The four-eye butterflyfish, *C. capistratus*, occurs exclusively in the Caribbean, from Florida to Venezuela (Kuitert, 2002; Floeter *et al.*, 2008), while the banded butterflyfish, *C. striatus* is widely distributed from the Caribbean southwards to the rocky reefs of the southern Brazilian coast (Carvalho-Filho, 1999; Floeter *et al.*, 2008). In the Caribbean, these two species co-occur in most locations, although with different abundances and preferred microhabitats within reefs (Neudecker, 1985; Pitts, 1991).

Chaetodon capistratus is the most common and better studied species in the Caribbean (Birkeland & Neudecker, 1981; Lasker, 1985; Findley & Findley, 1989; Pitts, 1991), while *C. striatus* is less common and thus, have received much less attention. Previous assessments on the diet of the two species reported a diverse set of

invertebrates items in their stomachs, indicating them as possible generalist feeders, with octocorals found in the diet *C. capistratus* but not in *C. striatus* (Randall, 1967; Pitts, 1991). However, none of these studies have included comparisons on feeding bite rates.

This is the first study that compares feeding ecology of the sister-species *C. capistratus* and *C. striatus* with the combination of different methodologies, such as foraging behaviour (selectivity for microhabitats; bite rates), dietary composition, and nutritional condition. The combination of these data allows us a better understanding about *C. capistratus* and *C. striatus* feeding ecology.

METHODS

Fieldwork was carried out in the coral reefs around La Parguera, Puerto Rico (17°56'N, 67°01'W) in March 2011 (mean sea surface temperature – SST: 27°C). Underwater observations of *Chaetodon capistratus* and *C. striatus* were conducted between 9:00 and 16:00 h, with approximately 50 hrs of sampling effort in depths ranging between 18–25 m through SCUBA diving. Foraging behavior was quantified by following individual fishes (focal animal methodology; Lehner, 1996) for 3 min periods and counting the number of bites taken from each category of the benthic coverage (see details below). Individuals were selected haphazardly, totalizing 60 *C. striatus* and 30 *C. capistratus* observed. To minimize the risk of resampling the same individual, the observers moved through the sampling site to search for other individuals (Birkeland & Neudecker, 1981). We waited for a few minutes before started counting bites, in order to allow the fish to get used to the diver. Fishes were then followed at a discreet distance (1–3 m) where they did not appear to be affected by the observer (Birkeland & Neudecker, 1981; Alwany *et al.*, 2003). Only adult individuals were sampled (>10 cm) to ensure that results were not biased by potential ontogenetic changes in feeding.

The photoquadrat method (Preskitt *et al.*, 2004) was used to estimate the relative abundance of each substratum type. Five random photos (covering an area of 40 x 60cm; Krajewski & Floeter, 2011) were taken in an area of approximately 2 m x 2.5 m (5 m²) around the last bite taken by the fish being sampled (i.e. the end of each 3 min observation).

Diet

A total of 55 specimens (25 *Chaetodon capistratus* and 30 *C. striatus*) were collected using hand spear and total body sizes were measured (total length – TL). Stomach contents were immediately stored after collection in tubes with ethanol. Food items were identified under a stereomicroscope to the lowest possible taxonomic category and listed. The volume of each item was measured in a 1 mm high square-shaped transparent dish with a scale in millimetres underneath so that the area corresponded to the volume. The relative importance of each item was analysed through the Feeding Index (IAi), which combines frequency of occurrence and volume of each item, in relation to the total (Kawakami & Vazzoler, 1980). Contents were classified as “*digested organic matter*” when elements such as spicules, nematocysts, setae were found mixed within the organic matter. When we observed nematocysts in high abundance among the organic matter we classified them into Cnidaria Hexacorallia Actiniaria or Cnidaria Hexacorallia Zoantharia categories. However, it was not possible to distinguish the nematocysts from Cnidaria Hexacorallia Corallimorpharia and Scleractinia (Mariscal, 1974), so they are referred as Cnidaria Hexacorallia “Corallim/Scle”. When no identifiable elements were found, it was classified as “unidentifiable”. Taxonomic experts were consulted to confirm or provide identification of items.

In order to assess feeding activity, the degree of fullness of each stomach was recorded in the following categories: < 25%; 25–50%; 50–75% and >75%.

Nutritional condition

RNA:DNA ratio is a physiological index that is sensitive to changes in dietary status of organisms (Buckley & Szmant, 2004; Behrens & Lafferty, 2007). This index has been broadly employed once that RNA protein synthesis varies with metabolic demand, i.e. fluctuates in response to food availability (quality) while DNA content per cell is fixed (Calderone *et al.*, 2001; Chicharo & Chicharo, 2008).

In order to determine RNA:DNA ratios, white muscle of fishes was stored in RNALater (Quiagen) solution immediately after sampling and kept in -20°C freezer. Nine samples of *Chaetodon capistratus* and 21 of *C. striatus* were thawed to determine RNA and DNA concentrations of each sample using ethidium bromide fluorescence

(Bentle *et al.*, 1981, modified by Dahlhoff & Menge, 1996). Tissue samples of 100 mg were weighted and homogenized in 10 volumes of 2 mol NaCl replicates. The samples were centrifuged for 1 min, and then 5 µl of each sample was transferred into a microplate adding 0.14 µl of solution containing 0.005 mg ethidium bromide and 0.15 mg proteinase K at 37°C for 60 min. After initial incubation, 100 µl of buffer (80mm Tris-HCl, pH 8.0 at 20°C) was added to the process and then measured fluorescence at 365 nm excitation and 590 nm emission using a SpectraMax M5 spectrofluorometer (Molecular Devices). The fluorescence due to RNA and DNA was determined by sequential digestion of each nucleic acid using first 5 µl RNase I (SIGMA) followed by 60 min incubation and then 5 µl DNase I (SIGMA) followed by 30 min incubation. Last, RNA and DNA concentrations was estimated from a standard curve calculated by measuring the fluorescence of know quantities of RNA and DNA.

Fish density

To evaluate the abundance of *Chaetodon capistratus* and *C. striatus* in La Parguera, online published data was obtained from: http://www8.nos.noaa.gov/biogeopublic/query_fish.aspx. Data was collected by belt transects of 100 m² (25 m X 4 m).

Data analysis

The benthic cover in each photoquadrat was classified into 11 microhabitat categories: six algae-dominated types (epilithic algal matrix [EAM], crustose, foliose, leathery, corticated, articulated calcareous), Anthozoa (Octocorallia, Zoantharia, and Scleractinia), and other microhabitats (e.g. porifera and sand). To analyze the photographs we used the software Coral Point Count with Excel Extension (CPCe v3.5 - Kohler & Gill, 2006), where 20 random points on each photograph was added to estimate the relative cover of each microhabitat. It is worth noting that even though we observed fishes biting at these microhabitats, we could not see exactly which small item was ingested, i.e. cryptofauna associated to the epilithic algal matrix or sand (Kramer *et al.*, 2012).

Ivlev's Electivity Index ("E" in Krebs, 1989) was used to evaluate feeding selectivity, comparing what microhabitats are used for feeding with respect to their availability. The E values ranges from -1 to 1, in which values close to 1 indicate

preferences and values close to -1 indicate avoidance (Krebs, 1989). Preference or avoidance were tested with a null model that generated expected values for “E”, taking into account the number of bites and the availability of each benthic item. The total number of bites per individual was randomized across benthic categories (1,000 randomizations) and then employed to obtain a null distribution of E in the absence of selectivity. Confidence limits were determined using the percentile method (Manly, 1997) and preference or avoidance was considered statistically significant if the observed “E” was higher or lower, respectively, than 95% of the randomized datasets. The null model was generated using the R software (R Development Core Team 2012).

Feeding rates, fish density and RNA:DNA ratio was compared between species with Student’s t test (Zar, 1999).

RESULTS

Mean total length of *Chaetodon capistratus* individuals was 10.4 cm (\pm 1.05 SD) and *C. striatus* 13.9 cm (\pm 0.8 SD). *Chaetodon capistratus* had significant higher mean bite rate and density (bite rate: 3.36 ± 0.38 SE; density: 3.1 ± 0.08 SE) then *C. striatus* (bite rate: 1.38 ± 0.16 SE; density: 1.5 ± 0.7 SE) (Figure 1).

Stomach contents of *C. capistratus* had 24 items while *C. striatus* presented 30 different items (Table 1). Both species basically ingest similar items, reflecting in similar values of Feeding Index for “Corallim/Scle” (29.7% and 32.4%), and polychaetes “other” (22.9% and 22.8% for *C. capistratus* and *C. striatus*, respectively). However, other items seemed also important for *C. capistratus*, such as Cnidaria Octocorallia, with 9.3% of Feeding Index, represented by 48% of frequency of occurrence and Hexacorallia Zoantharia, with 19.8% of Feeding Index, represented by 28% of frequency of occurrence. These items had less importance in *C. striatus* diet (< 0.5% of Feeding Index; Table 1).

When analysing all items, 74.5% of the Feeding Index of *C. capistratus* was based on cnidarians, while *C. striatus* had 56% of the Feeding Index on Polychaetes. The degree of fullness for both species was mostly between 50–75%.

At La Parguera, both species forage on different microhabitats. While *C. capistratus* foraged much more on Anthozoa Scleractinia (31.4% of bites) and Octocorallia (33.1%), *C. striatus* bites were proportionally higher at EAM (44.2%) and

sand (14.3%). These two microhabitats are also common at this site, but only EAM was significantly and positively selected compared to the null expectation (Figure 2).

There were no significant differences between both species nutritional condition. Results of RNA:DNA ratio were 2.06 and 2.6 for *C. capistratus* and *C. striatus* respectively.

DISCUSSION

Butterflyfishes have been intensely studied in tropical reefs of the Indo-Pacific where the diversity of this family is high and the association of some species with corals make them highly specialized. Some studies included specialists and more generalists species (Pratchett, 2005; Gregson *et al.*, 2008; Lawton *et al.*, 2011), but none of these studies have focused specifically on sister-species, which makes evolutionary comparisons more interesting.

At La Parguera reefs, in Puerto Rico, the sister-species, *C. capistratus* and *C. striatus* occur sympatrically. To better understand the feeding habits of these generalist butterflyfishes, the combination of three complementary approaches (foraging behaviour, stomach content analysis, and nutritional condition) were employed. While the first method is based in field observations, the second identifies cryptic dietary species that was not possible to detect while observing fish feeding (Nagelkerken *et al.*, 2009). Finally, the nutritional condition revealed the physiological status of the species.

Chaetodon capistratus was twice more abundant than *C. striatus*, supporting results found by Pitts (1991) in Bahamas (five times more abundant). Patterns of comparative low abundance for *C. striatus* in the Caribbean were also reported by Neudecker (1985) in St. Croix, US Virgin Islands, where in fact this species was considered rare. Along the Brazilian coast, where *C. capistratus* is absent, *C. striatus* is the most abundant chaetodontid (Ferreira *et al.*, 2004). At some localities in the subtropical reefs it co-occurs with *C. sedentarius* (not from the subgenus *Chaetodon*) and also, it can co-occur with *C. ocellatus* in tropical reefs. However, *C. ocellatus* is virtually rare in the Brazilian coast being more abundant in the Oceanic Islands.

While foraging, *C. capistratus* bite rate was significantly higher than *C. striatus*. Previous studies in St. Croix Island in the Caribbean reported mean bite rate/min about

5.9±1.9 (SE) and 5.4±1.6 (SE) for *C. capistratus* (Birkeland & Neudecker, 1981; Neudecker, 1985; respectively), revealing that values for La Parguera were slightly lower: 3.36 ± 0.38 (SE).

In the Southeastern Brazilian coast, Bonaldo *et al.* (2005) found a mean bite rate range of 1.5–2.3 bites/min for *C. striatus*, a result similar to our data from La Parguera (1.38 bites/min). Along the Brazilian coast values can range from 1.7 to 3.5 (authors unpubl data). It seems that when *C. striatus* does not co-occurs with other species from the subgenus *Chaetodon*, it may have higher feeding rates.

Chaetodon capistratus had 64.5% of its bites on anthozoans, while *C. striatus* had 23.8%. On the other hand, *C. striatus* had 58.5% of its bites on EAM and sand microhabitats, while *C. capistratus* had only 24.4% of its bites on those microhabitats.

In regard to diet, the similarity of items found in the stomach contents of both species was close to what was described by Pitts (1991). Unfortunately, this study by Pitts (1991) did not include field observations on bite rate. An example of overlap of items is the presence of anthozoans and polychaetes in both species stomach contents. Previous studies pointed out that 93.6% and 89% of *C. capistratus* bites were on anthozoans (Birkeland & Neudecker, 1981; Neudecker, 1985; respectively) and 73.5% of the stomach contents confirmed the presence of anthozoans (Neudecker, 1985). Yet, we also found that *C. capistratus* ingested mainly anthozoans (64.5% of bites; 74.6% IAI). Despite the high proportion of bites on Octocorallia (33.1%), the stomach contents of *C. capistratus* had only 9.3% of Feeding Index on this item. This is an example of the importance of combining knowledge of field observations and analysis of stomach contents to understand the feeding behaviour of a species, once each approach separately may present different results.

Chaetodon striatus most important dietary item was polychaetes (45.7% of volume = 56% IAI). Randall (1967) also found similar results (58.7% of volume). As the microhabitats with most bites were EAM (44.2%) and sand (14.3%), it is likely that polychaetes are using them as refuge. Also, studies with eight populations of *C. striatus* stomach contents along the Brazilian coast (A Liedke, Universidade Federal do Paraná, unpubl data) resulted in similar dietary items, being polychaetes the most important food item.

Moreover, as described by Pitts (1991) both species seem to have some habitat partition. While *C. capistratus* feeds much more at the centre of reef patches, mostly on octocorals and scleractinians, *C. striatus* was usually found at the borders of the reef patches, closer to the interface of the reef and sand (Liedke & Floeter, pers. obs). In fact, we observed that *C. striatus* takes most of its bites in sand, turf, and algae dominated substrates (69.5% of bites) where invertebrates are probably abundant. The bulk of stomach contents for *C. striatus* were sessile invertebrates (86% of the volume are polychaetes and cnidarians).

Although both species are broadcast spawners and have similar pelagic larval durations (33-45 days – B Victor and L Vigliola pers. comm.), the fact that *C. striatus* have a higher feeding plasticity (reflected by the higher number of items in its stomach contents) may explain its larger distribution range, allowing it to reach the southern Brazilian coast. Moreover, as *C. capistratus* most important dietary item is Anthozoa Octocorallia and this item does not represent the bulk of the reef physiognomy southwards of the Amazon mouth. This fact probably is a restrictive factor to its distribution, being this species endemic to the Caribbean.

The variety and different proportion of dietary items, as well as different bite rates found for the sister-species apparently did not influence their nutritional condition. The results in this study indicate that both species may not be under physiological stress, such as starvation, which could be considered if values were lower than one (Kono *et al.*, 2003; Beherens & Lafferty, 2007).

As *C. capistratus* is the commonest butterflyfish in the Caribbean with high abundance, most studies were done with this species (Birkeland & Neudecker, 1981; Lasker, 1985). During our field expedition at La Parguera, we noted that *C. capistratus* occurs in high frequency both in shallow and outer reefs. Differently, *Chaetodon striatus* are found only in the outer reefs, at about 18-25m depth, which suggests that depth is a major constraint for studies of this species in the Caribbean.

The sister-species, *Chaetodon capistratus* and *C. striatus* ingest similar dietary items, however, in different proportions. The feeding microhabitats used for browsing were different, while *C. capistratus* had most of its bites (and stomach contents) in anthozoans, this species rejected microhabitats such as sand, foliose algae and porifera. On the other hand, *C. striatus* selected the microhabitat covered with EAM to browse

small preys, such as polychaetes and anthozoans “Corallim/Scle” (although much less bites in anthozoans *per se* when compared to *C. capistratus*).

Although the broad quantities of dietary items found, the generalist feeding behaviour with high presence of chemically defended polyps (Suchanek & Green, 1981) and very small invertebrates (e.g. crustaceans) also reveals that some visual and mechanical specialization (e.g. teeth, jaws, swimming ability – Motta, 1989; Ferry-Graham *et al.*, 2001; Leis & Yerman, 2012) is still present and in use by these “generalist zoobenthivores”.

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Legends

Table 1. Frequency of occurrence (%FO), Volume (%V) in the diet and Feeding Index (%IAi) of dietary items of *Chaetodon capistratus* and *C. striatus* in La Parguera, Puerto Rico.

Figure. 1. A) Density; B) Mean bite rates; C) RNA:DNA ratio for of *C. capistratus* (grey bars) and *C. striatus* (white bars) at Puerto Rico. * significantly different ($p < 0.05$)

Figure. 2. Percentage of benthic cover (white bars) and bites (black bars) for *C. capistratus* and *C. striatus*. Algae: EAM: epilithic algal matrix; MCRO: crustose, MFOL: foliose, MCOR: leathery, MCT: corticated, MCA: articulated calcareous; Anthozoans: ANT OCT Octocorallia, ANTZOA Zoantharia, ANT SCL Scleractinia; POR: Porifera and Sand. * indicates IVLEV significantly different than the null model (< 0.05).

Table 2. Frequency of occurrence (%FO), Volume (%V) in the diet and Feeding Index (%IAi) of dietary items of *Chaetodon capistratus* and *C. striatus* in La Parguera, Puerto Rico.

n° of stomachs n° of items		<i>C. capistratus</i>			<i>C. striatus</i>		
		25			30		
		24			30		
		%FO	%V	%IAi	%FO	%V	%IAi
Algae		4.0	<0.1	<0.1	23.3	0.5	0.2
Cnidaria Hydrozoa					10.0	0.4	0.1
Cnidaria -	Octocorallia	48.0	7.1	9.3	6.7	0.1	<0.1
	Hexac. Zoantharia	28.0	25.9	19.8	3.3	1.0	0.1
	Hexac. Corallim./ Scle.	40.0	27.3	29.7	60.0	31.8	32.4
	Hexac. Other	28.0	20.7	15.8	33.3	17.6	9.9
Annelida -	Sabellidae	16.0	0.2	0.1	76.7	20.4	26.6
Polychaeta	Serpulidae	44.0	0.7	0.8	56.7	6.9	6.6
	Cirratulidae				6.7	1.2	0.1
	Eunicidae				6.7	0.5	0.1
	Syllidae	32.0	1.1	0.9	6.7	0.1	<0.1
	Terebellidae	4.0	0.2	<0.1	3.3	0.2	<0.1
	Other	84.0	11.1	22.9	93.3	16.4	22.8
Arthropoda	Amphipoda	12.0	0.1	<0.1	10.0	<0.1	<0.1
- Crustacea	Isopoda				3.3	0.1	<0.1
	Decapoda				6.7	0.1	<0.1
	Other	12.0	<0.1	<0.1	10.0	<0.1	<0.1
	Mollusca	24.0	0.2	0.1	33.3	1.3	0.4
Eggs	Crustacea				6.7	0.1	<0.1
	Other	8.0	<0.1	<0.1			
Others*		4.0	0.1	<0.1	23.3	0.1	<0.1
Inorganic elements**		16.0	0.8	0.1	60.0	0.5	0.2
Digested Organic Matter***		4.0	4.3	0.5			
Unidentified		16.0	0.1	<0.1	36.7	0.8	0.5

*Composed by Foraminiferida, Ectoprocta, Porifera, Platyhelminthes, Nematomorpha, Echinodermata (Ophiuroidea and Holothuroidea) and Angiosperma.

**Composed by fragments of silica and calcareous skeletons.

***Composed by nematocysts, spicules and setae.

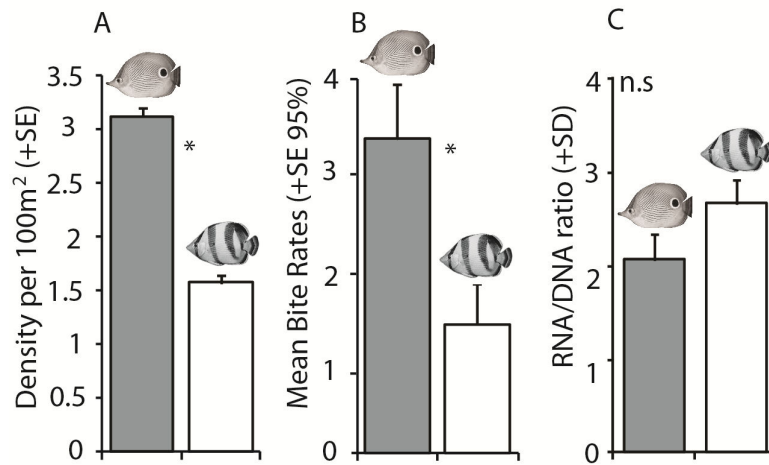


Figure. 1. A) Density; B) Mean bite rates; C) RNA:DNA ratio for of *C. capistratus* (grey bars) and *C. striatus* (white bars) at Puerto Rico. * Significantly different (p<0.05)

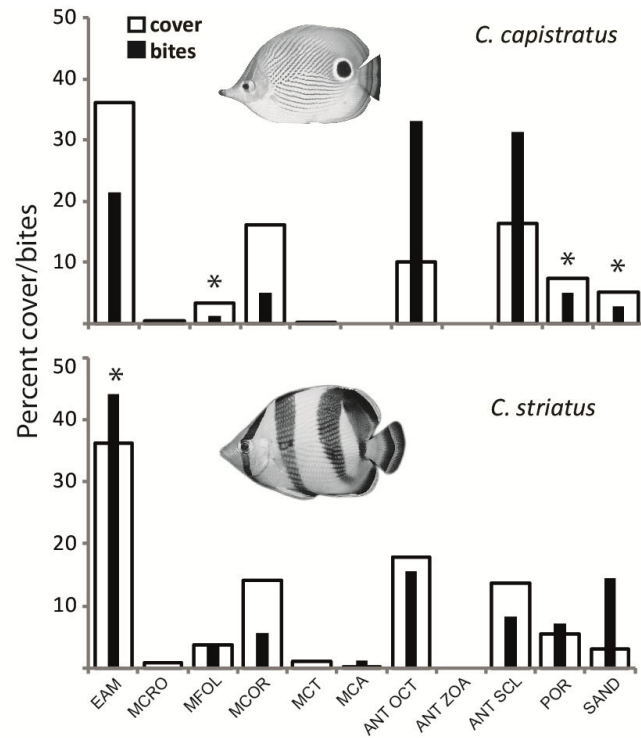
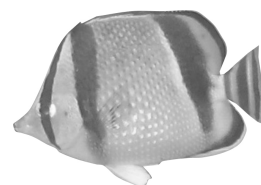


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Artigo III



**Feeding ecology of *Chaetodon humeralis* (Gunter 1860) in the
Sea of Cortez, Mexico**

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Feeding ecology of *Chaetodon humeralis* (Gunter 1860) in the Sea of Cortez, Mexico

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Abstract

Butterflyfishes are broadly studied all over the world due to its intrinsic relation with the environment. The majority of these studies have been carried out in the Indo Pacific, in the Caribbean and in the Red Sea, but little is known about the threebanded butterflyfish, *Chaetodon humeralis*, the only species of this genus in the Tropical Eastern Pacific. Here we present the first information on its foraging behavior and diet in the Sea of Cortez. The aim of this study was to evaluate: 1) feeding bite rates; 2) foraging selectivity for microhabitats; 3) abundance; 4) diet; and 5) nutritional condition. Feeding observations were conducted with 57 randomly selected individuals and gut contents from 23 collected individuals. Ivlev's Electivity Index with the random generated confidence interval did not show selectivity or rejection for any microhabitat used for foraging. The Feeding Index was composed mostly by cnidarians (40.8%), polychaetes (40.1%) and Algae (16.6%). RNA:DNA ratio had a mean of 2.65 (+1.18 SD), pointing out that *C. humeralis* have a good nutritional condition in this environment. *Chaetodon humeralis* exhibited a high diet breadth in the Sea of Cortez (even including algae in its diet) and may be considered a generalist benthivore.

Keywords: Baja California, Gulf of California, Tropical Eastern Pacific, butterflyfish, Chaetodontidae.

Introduction

The majority of studies of butterflyfishes (Chaetodontidae) have been carried out in the Indo-Pacific (Hobson 1974; Irons 1989; Tricas 1989; Pratchett 2005), in the Caribbean (Birkeland & Neudecker 1981; Lasker 1985; Pitts 1991), and in the Red Sea (Alwany *et al.* 2003, 2007). Although their feeding habits have attracted the attention of researchers due the intrinsic relation with the environment (Pratchett & Berumen 2008), little is known about species in the Tropical Eastern Pacific.

The threebanded butterflyfish, *Chaetodon humeralis*, is the commonest butterflyfish and the only species of this genus in the Tropical Eastern Pacific (Thomson *et al.* 2000). All the other species from the subgenus Chaetodon inhabit the Western Atlantic Ocean (Kuitert 2002; Fessler & Westneat 2007; Bellwood *et al.* 2010). *Chaetodon humeralis* sister-species is *C. ocelatus* and its sister clade are formed by *C. capistratus* and *C. striatus* (Bellwood *et al.* 2010). The threebanded butterflyfish distribution ranges from the central to south Sea of Cortez and Pacific Coast of Baja California to Peru, including the oceanic islands of Cocos, Malpelo and Galapagos Island (Thomson *et al.* 2000; Humann & Deloach 2004). They are usually found in pairs or small groups (Thomson *et al.* 2000).

This species is common at depths from 3–12 m but it can be found even below 55m (Thomson *et al.* 2000). There is no color change during ontogeny. The few studies that include *C. humeralis* are almost exclusively based on community structure in the Sea de Cortez (Rodríguez-R *et al.* 1994; Rodríguez-R *et al.* 2005; Galván-Magaña *et al.* 1996; Aburto-Oropeza & Balart 2001) and Galápagos (McCosker & Rosenblatt 2010). Therefore, none of them describes the feeding behavior of *Chaetodon humeralis*.

The feeding ecology of a species is an important aspect of its biology. The relationship between food availability, food quality and resulting physiological state will likely reflect in its distribution and abundance and also its foraging behavior.

Here we present the first study on the feeding ecology of *C. humeralis*. The aim of this study was to evaluate: 1) feeding bite rates; 2) foraging selectivity for microhabitats; 3) abundance; 4) diet; and 5) nutritional condition. Moreover, we will compare these results with other close related species of the subgenus Chaetodon.

Material and Methods

Study area

Fieldwork was carried out in two rocky reefs in the Sea of Cortez, Mexico. The first one is located at the mainland side, in San Carlos, Sonora, in the Central Gulf (27°56'N, 111°05'W) and was sampled in December 2010 (SST: 22°C). The second one is located in Baja Peninsula, in La Paz, Baja California Sur, the Lower Gulf (23°59'N, 109°49'W). This site was sampled in May 2011 (SST: 24°C) (Fig. 1).

Foraging behavior

Underwater observations were conducted only in La Paz, between 9:00 and 16:00 h, with approximately 25 h of sampling effort in depths ranging between 2–4m. Foraging behavior was quantified by following individual fishes (focal animal methodology; Lehner 1996) for 3-min periods and counting the number of bites taken from each category of the benthic coverage (see details in Data analyses). Fifty-seven individuals were selected haphazardly. To minimize the risk of resampling the same individual, the observers moved through the sampling site to search for other individuals (Birkeland & Neudecker 1981). We waited for a few minutes before we started the counting, in order to allow the fish to get used to the diver. Fish were then followed at a discreet distance (1–3m) where fish did not appear to be affected by the observer (Birkeland & Neudecker 1981; Alwany 2003). We sampled only adult individuals (>10cm), to ensure that results were not biased by potential ontogenetic changes in feeding.

We used photoquadrat method (Preskitt *et al.* 2004) to estimate the relative abundance of each substratum type. Five random photos (covering an area of 40 x 60cm; Krajewski & Floeter 2011) were taken in an area of approximately 2m x 2.5m (5m²) around the last bite taken by the fish being sampled (i.e. the end of each 3-min observation).

Diet

Fishes were collected using hand spear and total body sizes were measured immediately (total length – TL). Gut contents were immediately stored after collection in

tubes with ethanol. Food items were identified under a stereomicroscope to the lowest suitable taxonomic category and listed. The volume of each item was measured in a 1mm high square-shaped transparent dish with a scale in millimetres underneath so that the area corresponded to the volume. The relative importance of each item was analysed through the Feeding Index (IA_i), which combines frequency of occurrence and volume of each item, in relation to the total (Kawakami & Vazzoler 1980). We identified all possible dietary items found in *C. humeralis* gut contents. When we found masses of organic material that seemed to be unidentifiable, they were analyzed under microscope in search for clue elements. Contents were classified as “*digested organic matter*” when elements such as spicules, nematocysts, setae were found mixed within the organic matter. When we observed only nematocysts, in high abundance among the organic matter we classified them into Cnidaria Hexacorallia Zoantharia category. However, we could not distinguish the nematocysts from Cnidaria Hexacorallia Corallimorpharia and Scleractinia (Mariscal 1974), so we referred to them as Cnidaria Hexacorallia Corallim./Scle. When no identifiable elements were found, it was classified as “unidentifiable”. Several taxonomic experts were consulted to confirm or provide identification of dubious items. Data was arranged in 23 groups, as shown in the results.

Nutritional condition

RNA:DNA ratio is a physiological index that is sensitive to changes in dietary status of organisms (Buckley & Szamant 2004; Behrens & Lafferty 2007). This index has been broadly employed once that RNA protein synthesis varies with metabolic demand, i.e. fluctuates in response to food availability (quality) while DNA content per cell is fixed (Calderone *et al.* 2001; Chícharo & Chícharo 2008). In order to determine RNA:DNA ratios, we stored white muscle of eleven fishes in RNALater (Quiagen) solution immediately after sampling and kept in -20°C freezer. Samples were thawed and we first determined RNA and DNA concentrations of each sample using ethidium bromide fluorescence (Bentle *et al.* 1981, modified by Dahlhoff & Menge 1996). We weighted samples to 100 mg and homogenized them in replicates with 10 volumes of 2 mol NaCl. The samples were centrifuged for 1 min, and then 5 µl of each sample was transferred into a microplate adding 0.14 µl of solution containing 0.005 mg ethidium bromide and 0.15 mg proteinase K at 37°C for 60 min. After this initial incubation, we

added 100 μ l of buffer (80mm Tris-HCl, pH 8.0 at 20°C) and then measured fluorescence at 365 nm excitation and 590 nm emission using a SpectraMax M5 spectrofluorometer (Molecular Devices). We determined fluorescence due to RNA and DNA by sequential digestion of each nucleic acid using first 5 μ l RNase I (SIGMA) followed by 60 min incubation and then 5 μ l DNase I (SIGMA) followed by 30 min incubation. Last, we estimated RNA and DNA concentrations from a standard curve calculated by measuring the fluorescence of known quantities of RNA and DNA.

Fish density

Underwater surveys were carried out with SCUBA using standard underwater visual belt transect survey methods (Harmelin-Vivien *et al.* 1985). The authors were experienced at visually surveying fish assemblages and practiced at estimating fish lengths accurately (Aburto-Oropeza & Balart 2001; Sala *et al.* 2002). From 1998 to 2010, five replicate 50 \times 5 m transects were surveyed at 5 locations in La Paz region and one in San Carlos. Transects were placed randomly on the substratum. A diver swam each transect at a constant speed (about 15 min per transect), counting all *C. humeralis* within 2.5 m to either side of the center line (250 m² transect area). In order to compare this data with the abundance of *Cheatodon* species from the Atlantic we transformed the data to 100m².

Data analyses

The benthic cover in each photo was classified into seven microhabitats categories: epilithic algal matrix [EAM], crustose algae, foliose algae, leathery algae, corticated algae, Anthozoa Scleractinia and sand. To analyze the photographs we used the software Coral Point Count with Excel Extension (CPCe v3.5 - Kohler & Gill 2006), where 20 random points on each photograph was added to estimate the relative cover of each microhabitat.

Ivlev's Electivity Index ("E" in Krebs 1989) was used to evaluate feeding selectivity, comparing which microhabitats are used for feeding with respect to their availability. The E values ranges from -1 to 1, in which values close to 1 indicate preferences and values close to -1 indicate avoidance (Krebs 1989). We tested for preference/avoidance with a null model that generated expected values for "E", taking

into account the number of bites and the availability of each benthic item. The total number of bites per individual was randomized across benthic categories (1,000 randomizations) and then employed to obtain a null distribution of E in the absence of selectivity. Confidence limits were determined using the percentile method (Manly 1997) and preference or avoidance was considered statistically significant if the observed “E” was higher or lower, respectively, than 95% of the randomized datasets. The null model was generated using the R software (R Development Core Team 2012).

One-way ANOVA was used to compare feeding rates, RNA:DNA ration, and fish density values found for *Chaetodon humeralis* in the Sea of Cortez with two species from the subgenus in the Western Atlantic (*C. striatus* and *C. capistratus*). When significant, post-hoc tests (SNK) were carried out to investigate such differences. Data met the assumptions for normality of residuals and homogeneity of variances (Zar 1999; Underwood 1997).

Results

Chaetodon humeralis mean bite rate per min was 0.85 ± 0.15 (SE). The two most abundant available microhabitats were sand (30.3%) and turf algae (29.7%). *Chaetodon humeralis* bites were mostly in sand (41.5%), leathery algae (40.8%) and turf algae (16.3%) (Fig 2). Ivlev's Electivity Index (E) with randomly generated confidence intervals did not show selectivity or rejection for any particular microhabitat used for foraging. All results fell within the expected value generated by the null model (Appendix 1).

We collected a total of 23 specimens (4 in San Carlos and 19 in La Paz). Fishes had a total length between 12 and 18 cm and an average of 14.5 cm. The analysis of gut contents showed the presence of 23 different items. The frequency of occurrence and volume of all items as well as the Feeding Index (IA_i) are on Table 1.

The Feeding Index was composed by 40.8% by cnidarians, 40.1% by polychaetes and 16.6% by Algae (73% frequency of occurrence). Some of the algae found were: *Bryopsis* sp., *Ceramium* sp., *Chondracanthus*, *Erythrotrichia carnea*, *Gelidium crinale*, *Hypnea* sp., *Jania* sp., Naviculacea, *Padina* sp., *Polysiphonia* sp., *Sargassum* sp., *Sargassum vulgares*, *Sphacelaria*.

The analysis of RNA:DNA ratio (with samples from La Paz) resulted in a mean of 2.65 (+1.18 SD). *Chaetodon humeralis* abundance was 0.4 ± 0.14 SD ind/100m² in La Paz and 0.23 ± 0.05 SD ind/100m² in San Carlos. The mean abundance was 0.39 ± 0.009 SD individuals per 100m² (Fig 3).

Discussion

This is the first study that analyzed the foraging behaviour, dietary composition, and nutritional condition of *Chaetodon humeralis*. The combination of these data allows us a better understanding about *C. humeralis* feeding ecology.

Chaetodon humeralis bite rate is lower than other butterflyfishes from the Pacific (Gregson *et al.* 2008). However, when comparing to other species from the same evolutionary clade (subgenus *Chaetodon*), we observed that its bite rate is similar to *C. striatus* but lower than *C. capistratus* (Fig. 3). The similar bite rate may be related to the similar main ingested items (e.g. polychaetes) by *C. humeralis* and *C. striatus* that are considered a relatively high nutritional value item (Birkeland & Neudecker 1981).

Chaetodon humeralis had a diet composed of a variety of polychaetes and Cnidaria, similarly to *Chaetodon striatus* which is a generalist zoobenthivore (Liedke *et al.* in prep). However, *C. humeralis* also have a considerable amount of algae (IAi 16%) when compared to *C. striatus* and *C. capistratus* (where IAi <0.2, see Liedke *et al.* in prep). This is not a usual characteristic among butterflyfishes, once most species can be categorized as either 1) obligate coral-feeders, 2) facultative coral-feeders, 3) non coral-feeders or 4) generalists (Pratchett 2005). Nevertheless, there are some butterflyfishes in the West Pacific that also ingest algae in a considerable frequency, e.g. *C. ephippium*, *C. citrinellus* (Harmelin-Vivien 1983) and *C. argentatus* (Sano 1989).

Unfortunately, there is still no data on the diet or foraging behavior of the relatively less abundant *Chaetodon ocellatus*, which is *C. humeralis* sister-species. However, during our field work in the Western Atlantic, the very few individuals that were sighted were found close to the reef-sand interface, similarly to *C. humeralis* behavior.

The lack of foraging selectivity of *Chaetodon humeralis*, reinforce the fact that this species is a browser on vast variety of dietary items, with a generalist diet. This may be a response to the characteristics of the environment in the Sea of Cortez. During our

observations, we noted that *C. humeralis* did not look for food in two microhabitats, crustose and foliose algae. Despite the high feeding rate in leathery algae, this was not significant. As the most important items that we found in the stomach contents were polychaetes and cnidarians, it is likely that *C. humeralis* find them in the microhabitats where they mostly invested: turf algae, leathery algae and sand.

The present study revealed the presence of items with high frequency of occurrence but representing low volume. Therefore, this reflects in a low Feeding Index (e.g. crustaceans and egg mass). These items are probably an important complementary resource, due to their high energetic value (Birkeland & Neudecker 1981; Fyhn & Govoni 1995).

RNA:DNA ratio is lower in fishes under starvation (Dahlhoff 2004) and fishes can respond to changes in food availability in order of days (Olivar *et al.* 2009). The RNA:DNA ratio of *C. humeralis* showed to be similar to the two other butterflyfishes of the subgenus *Chaetodon* (ranging from 1.5 to 2.7 – *Chaetodon striatus* and *C. capistratus*; Fig. 3). This result provides evidences that all three species present behavioral and physiological adaptations to achieve good nutritional condition independently of the water temperature, reef system type and availability of dietary items.

We observed in field that *Chaetodon humeralis* is a species with moderate abundance in the Sea of Cortez. Also, when comparing the results of both sites, the abundance of this species decreases from southern reefs towards the reef located in the northern area of the Gulf. These results corroborates those found by Aburto-Oropeza & Balart (2001), and those described by Thomson *et al.* (2000) who observed that this species is found on the interface between shallow rocky reefs and sandy bottoms. In the rocky reefs of La Paz, we observed several pair and trios that were swimming close to each other forming an aggregation that had a total of 18 fishes, while in San Carlos, we only observed *C. humeralis* in pairs. Pair behaviour is common between butterflyfishes, and the formation of small groups can be a behaviour associated to sites where there are few other congeners (Kuitert 2002) and species have more availability of feeding items.

In relation to other Chaetodontidae, *Chaetodon humeralis* exhibited high breadth diet in the Sea of Cortez, including a good proportion of algae, thus could be considered a generalist benthivore, while the other two species of the subgenus *Chaetodon* (*C.*

striatus and *C. capistratus*) can be considered as generalist zoobenthivores (Liedke *et al.* in prep.). It would be interesting to investigate the feeding habits of *Chaetodon humeralis* along its distribution range, including the southernmost extreme to check if there is plasticity and/or feeding selectivity.

Acknowledgments

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Legends

Table 3. Frequency of occurrence (%FO), Volume (%V) in the diet and Feeding Index (%IAi) of dietary items of *Chaetodon humeralis* in the Sea of Cortez.

Figure 1. Map of the sampling sites in Mexico, on the Sea of Cortez (Gulf of California).

Figure 2. Percentage of benthic cover (white bars) and bites (black bars) of each microhabitat. Algae: turf; MCRO: crustose, MFOL: foliose, MCOR: leathery; ANT SCL Anthozoans Scleractinia; and Sand.

Figure 3. A) Mean bite rates; B) density and, C) RNA:DNA ratio for of *C. capistratus*, *C. striatus* at Puerto Rico and Florianópolis (the southernmost limit for *C. striatus*) and *C. humeralis*. Letters indicate statistical grouping differences Newman-Keuls SNK *post hoc* test.

Figure 4. Electivity Index (*E*) for *Chaetodon humeralis* on different microhabitats. Circles represent the *E* value; gray bars represents 95% of confidence intervals obtained with the null model (see Methods). Algae: turf; MCRO: crustose, MFOL: foliose, MCOR: leathery; ANT SCL Anthozoans Scleractinia; and Sand.

Table 4. Frequency of occurrence (%FO), Volume (%V) in the diet and Feeding Index (%IAi) of dietary items of *Chaetodon humeralis* in the Sea of Cortez.

N° of items		23		
Dietary components		%FO	%V	%IAi
Algae		73.91	9.54	16.21
Cnidaria Hydrozoa		17.4	0.3	0.1
Cnidaria - Anthozoa	Octocorallia	13.04	7.66	2.30
	Hexac. Zoantharia	4.35	2.29	0.23
	Hexac. Corallim./ Scle.	39.13	9.82	8.84
	Hexac. Other	52.17	23.74	28.48
Annelida - Polychaeta	Sabellidae	8.70	0.70	0.14
	Serpulidae	4.35	0.88	0.09
	Other	78.26	21.61	38.90
Arthropoda - Crustacea	Amphipoda	26.09	0.36	0.21
	Tanaidacea	8.70	0.05	0.01
	Cumacea	4.35	0.01	<0.01
	Cirripedia	4.35	0.02	<0.01
	Other	8.70	0.02	<0.01
Eggs	Mollusca	26.09	17.26	2.24
	Fish	4.35	0.40	0.04
	Other	4.35	1.54	0.15
Echinodermata	Echinoidea	8.70	0.24	0.05
Others*		8.70	1.23	0.25
Inorganic elements**		60.87	1.16	1.40
Unidentified		13.04	1.19	0.36

*Composed by fish scales

**Composed by fragments of silica and calcareous skeletons.

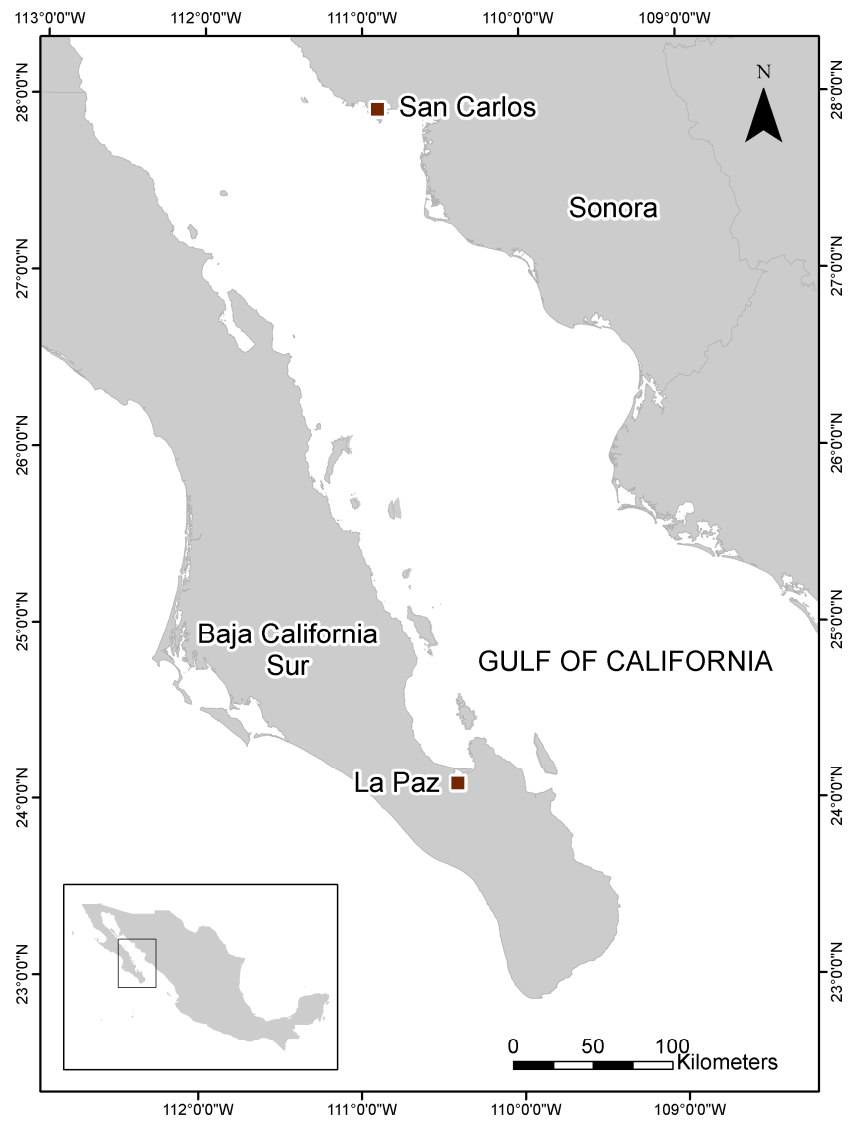


Figure 1. Map of the sampling sites in Mexico, on the Sea of Cortez (Gulf of California).

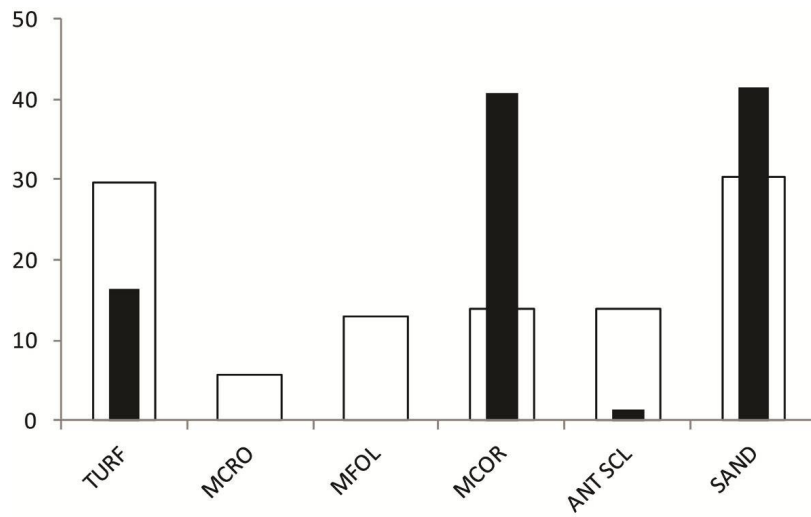


Figure 2. Percentage of benthic cover (white bars) and bites (black bars) of each microhabitat. Algae: turf; MCRO: crustose, MFOL: foliose, MCOR: leathery; ANT SCL Anthozoans Scleractinia; and Sand.

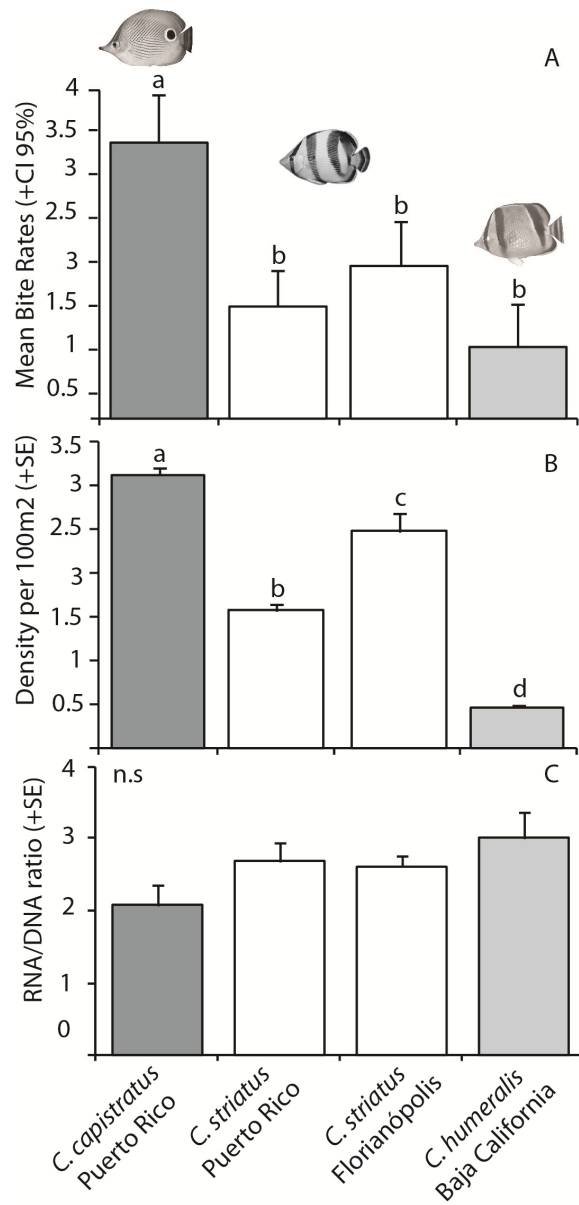


Figure 3. A) Mean bite rates; B) density and, C) RNA:DNA ratio for of *C. capistratus*, *C. striatus* at Puerto Rico and Florianópolis (the southernmost limit for *C. striatus*) and *C. humeralis*. Letters indicate statistical grouping differences Newman-Keuls SNK *post hoc* test.

Appendix

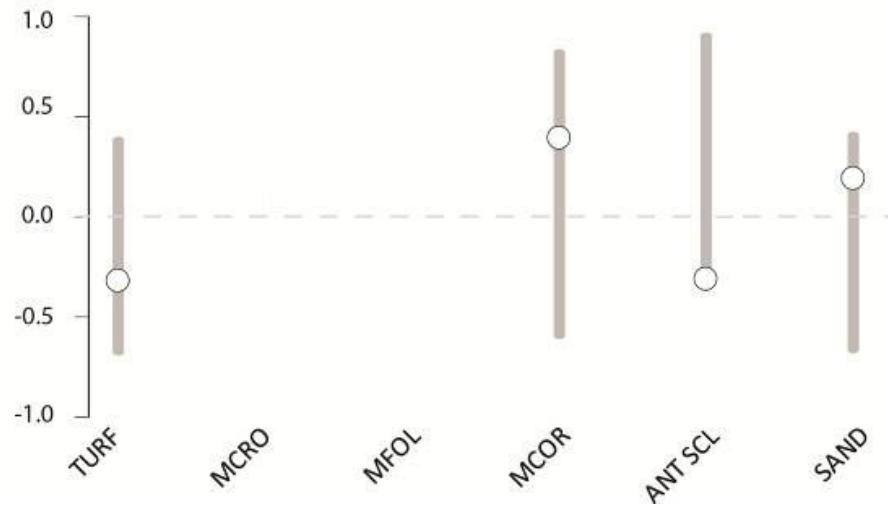
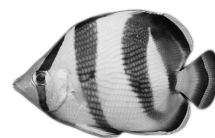


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Artigo IV

**Phylogeography of the banded butterflyfish *Chaetodon striatus*
along the Western Atlantic Ocean**

ANA M.R. LIEDKE, SERGIO R. FLOETER, GIACOMO BERNARDI

A ser submetido para “Marine Biology”

Phylogeography of the banded butterflyfish *Chaetodon striatus* along the western Atlantic Ocean

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Abstract

The knowledge of marine connectivity is an important tool for management of marine protected areas. *Chaetodon striatus* is a species that inhabit the Western Atlantic, from the Caribbean to southern Brazil. Its large distribution range includes different biogeographic provinces and barriers (e.g. outflow of Orinoco and Amazon rivers), as well as biotic and abiotic differences (e.g. reef type and water temperature). To test the genetic connectivity between populations along its wide distribution range, we sequenced three molecular markers (Dloop, cytB and Rag 2) from samples of seven localities. Our results indicate that there is a lack of population structure, and all populations have the same genetic diversity. Also, this species have had a recent population expansion around 80-100 thousand years, probably due to the Pleistocene climate changes associated with the enlargement of coastal shelf areas. These results are important to define conservations strategies.

Introduction

Studies with genetic connectivity of marine organisms had increased in the last decades (e.g. Jones, 2007) due to its importance to the effective conservation and management of marine resources (Palumbi et al., 2003; Palumbi, 2004). As many marine organisms are relatively sedentary as adults, the capacity of dispersing is primarily during the larval phase (Leis, 1991). The dispersal potential is also related to the egg type (pelagic vs. demersal) and pelagic larval duration (PLD). However, there

are studies in which exceptions were described, where larvae settled much closer than the potential for dispersal. Populations genetically structured, even with extensive larval duration have been documented (Planes et al., 1998; Almany et al., 2007; Jones, 2007).

The butterflyfishes are circumtropical, being present in all tropical and subtropical oceans. The vast majority of species inhabits the Indo-Pacific Ocean (Nelson, 2006). In the Western Atlantic Ocean there are a few species, mostly found in the Caribbean. The subgenus *Chaetodon* (Fessler and Westneat, 2007; Bellwood et al., 2010) is formed by two species pairs in the New World: *Chaetodon capistratus* and *C. striatus* and its sister clade *C. ocellatus* and *C. humeralis* (Bellwood et al., 2010). The last species inhabits the Tropical Eastern Pacific, while the remains are present in the Western Atlantic Ocean. The four-eye butterflyfish, *C. capistratus*, occurs exclusively in the Caribbean, from Florida to Venezuela (Kuitert, 2002; Floeter et al., 2008) being very abundant. *Chaetodon ocellatus* occurs in the Caribbean and northeast Brazil, but with low abundance. In reefs where it is the only butterflyfish inhabiting, it has a greater abundance, as it happens in two Brazilian oceanic Islands: Rocas Atoll and Fernando de Noronha (SRF pers com).

The banded butterflyfish, *C. striatus* is the species of this subgenus with the wider distribution, inhabiting from the coral reefs in the Caribbean to the rocky reefs on the southern Brazilian coast, more precisely in the State of Santa Catarina (Carvalho-Filho, 1999; Floeter et al., 2008). This large distribution range includes different biogeographic provinces, the Caribbean and Brazil (Floeter et al., 2008), and the Orinoco and Amazon outflow barrier, which separates both provinces (Rocha et al., 2008; Floeter et al. 2008) because it spans on 2300 km of the NE coast of South America (established around 11 Ma – Hoorn et al., 1995). Moreover, the water temperature changes with its geographical range (from 17° to 30°C – NOAA <http://www.nodc.noaa.gov/sog/cortad/>) according to the effects of marine currents.

Several reef fishes have wide distribution across the Western Atlantic. For most of these species, it still remains unclear if there is a continued genetic exchange between geographically distant populations and what types of barriers could be influencing. As an example, water temperature and reef type (e.g. coral cover vs rocky reef) can act synergistically separating populations of a species on the northern and southern (Santos et al., 2006). Also, it is known that peripheral populations may exhibit lower genetic

diversity as a consequence of smaller effective population sizes and isolation (Eckert et al., 2008).

The reef habitat has a discontinuity southwards Santa Catarina (~600 km), being this locality the southernmost limit of distribution for tropical marine fishes. Like other butterflyfish, *Chaetodon striatus* PLD is in between 33-45 days (33-45 days – B Victor and L Vigliola pers. comm.). Previous studies show that there are no differences in abundance along the distribution range (Liedke et al., unpublished).

Some studies investigated genetic connectivity of butterflyfishes along large distribution ranges (Craig et al., 2010; Lawton et al., 2011; DiBattista et al., 2012; Messmer et al., 2012). Even though some of these studies resulted in no genetic structure among populations (i.e. high connectivity) (Messmer et al. 2012) others show that, besides the large potential of larval dispersal, there are species with low genetic connectivity (Lawton et al., 2011).

The sea level fluctuation during the Pleistocene acted as an important factor on the distribution and population size of marine organisms, once that it has direct influence on the reef area size (Siddell, 2003). This fact is corroborated in some studies that detected a signal of changes on effective population size (Bellwood and Wainwright 2002).

In this study, we use three molecular markers, the rapidly evolving mitochondrial DNA (mtDNA) control region sequences (Dloop), cytochrome b and a nuclear gene, the recombinant activating gene 2 (Rag 2) to evaluate the following predictions: 1) the Orinoco and Amazon biogeographical barrier could separate populations between the Caribbean and Brazil; 2) the reef type and water temperature influence connectivity among populations; 3) the populations on the extremes of distribution follow the expectation of lower genetic diversity and; 4) *Chaetodon striatus* had a stable population effective size along time.

Methodology

Sampling and DNA extraction

Fishes were collected between January 2010 and March 2012 through SCUBA diving and snorkelling, using a hand spear. A total of 117 specimens were sampled, in seven localities along *C. striatus* distribution range: Puerto Rico 17°56'N, 67°01'W;

Tamandaré 8°44'S, 35°05'W; Salvador 12°S, 38°W, Abrolhos 17°58'S, 38°42'W; Trindade Island 20°30'S, 29°20'W; Arraial do Cabo 23°S, 42°W and Florianópolis 27°17'S, 48°22'W (Fig. 1).

Muscular tissue was immediately stored at ambient temperature in 95% ethanol. Tissues were digested overnight at 55 °C in 650µL of extraction buffer (400 mm NaCl, 10 mm Tris, 2mm EDTA, 1% SDS). We purified the DNA by standard chloroform extraction and isopropanol precipitation (Sambrook et al., 1989).

Polymerase chain reaction and DNA sequencing

Amplification of the 5' hypervariable portion of the mitochondrial control region was accomplished with nested PCR's using first the primers CB3L (5'GGCAAATAGGAARTATCATTC 3') and CR-E (5'CCT GAA GTA GGA ACC AGA TG) followed by ProL (CTA CCT CCA ACT CCC AAA GC) and CR-E. Thermal cycling in polymerase chain reactions (PCR) consisted of an initial denaturation step at 94°C for 45 sec, then 35 cycles of amplification (45 sec of denaturation at 94°C, 30 sec of annealing at 52°C with the first pair of primers and 54°C in the second, and 1 min of extension at 72°C) and a final extension of 5 min of 72°C. In addition, we amplified and sequenced segments of the mitochondrial cytochrome b (CytB) using the primers CB3H (GGC AAA TAG GAA RTA TCA TTC) and GLUDG-L (TGA CTT GAA RAA CCA YCG TTG) with the polymerase chain reactions as: an initial denaturation step at 94°C for 45 sec, followed by 35 cycles of 45 sec at 94°C, 45 sec at 54°C, and 1 min at 72°C and a final extension of 5 min of 72°C. The nuclear protein coding recombination-activating gene 2 (Rag 2) was amplified with the following primers Rag 2 1F (GAG GGC CAT CTC CTT CTC CAA) and Rag 2 9R (GAT GGC CTT CCC TCT GTG GGT AC). The thermal cycling was performed by an initial denaturation step at 94°C for 45 sec, followed by 35 cycles of 30 sec at 94°C, 1 min at 60°C, and 2 min at 72°C with a final extension of 5 min of 72°C. Each 13-µL reaction contained 5–50 ng of DNA, 10 mm Tris-HCl (pH 8.3), 50 mm KCl, 1.5 mm MgCl₂, 1.25 U of Taq DNA polymerase (PerkinElmer), 150 mm of each dNTP, and 0.3 mm of each primer. After purification following the manufacturer's protocol (Applied Biosystems), direct sequencing was performed with an ABI 3100 automated sequencer (Applied Biosystems) at University of California Berkeley. Sequencing was performed

in one direction only for all genes. The putative nature of each sequence was confirmed by BLASTN search.

Data analysis

We used the computer program Geneious 5.0 (Biomatters) to align the sequences. All analyses were performed with four datasets, each gene individually (Dloop, CytB and Rag 2) and both mtDNA concatenated.

Genetic summary statistics as nucleotide and haplotype diversity, Tajima's D (Tajima, 1983) and Fu's Fs (Fu, 1997) neutrality tests were estimated with all datasets for each population, as well as for all individuals in the programs Arlequin version 3.5.1.2 (Excoffier and Lischer, 2010) and DNAsp (Rozas et al., 2003).

Genealogical relationships among haplotypes were estimated with a median-joining haplotype network using Network 4.6.1.1. (Fluxus Technology Ltd. at www.fluxus-engineering.com). Population structure and gene flow were assessed with an analysis of molecular variance AMOVA (Excoffier et al. 1992) in the program Arlequin. For this analyses, data was grouped in different regions and alternative scenarios of geographic subdivision: first representing tropical (PR, PE, SAL and AB) and subtropical localities (RJ and SC), then a second one separating the Caribbean (PR), the tropical Brazil (PE, SAL, AB) and the subtropical localities (RJ and SC). Population pairwise F_{ST} comparisons were calculated using Arlequin software ($p < 0.05$).

The most appropriate model of nucleotide substitution for each dataset was estimated using the Akaike Information Criterion (AIC) as implemented in Modeltest 3.06 (Possada and Crandall 1998). In addition, to analyze population size dynamics through time we used Bayesian Skyline Plot method implemented on BEAST 2.0 (Drummond et al., 2005) with the concatenated dataset (mtDNA). This Bayesian approach incorporates the uncertainty in the genealogy by using MCMC integration under a coalescent model, where the timing of divergence dates provides information about effective population sizes through time. The evolutionary model suggested by Modeltest, a length chain of 10,000,000. The mutation rate used for Dloop used was 6.94 – 7.83% and CytB 2.36 – 2.67% (Bernardi and Lape, 2005; Domingues et al., 2005) per million of years.

Results

Our alignments resulted in three matrices of 397 bp, 791 bp and 750 bp for mitochondrial Dloop (119 individuals), cytochrome b (116 individuals) and Rag 2 (106 individuals). Both mtDNA concatenated resulted in 1188 bp. As we had only two individuals from Trindade Island, we only included in the network haplotype analyzes (Dloop and Rag 2).

The Dloop was the most variable segment of *C. striatus* samples. From all nucleotides, 184 was phylogenetically informative. We found Dloop segment had 111 haplotype (Table 1). A low level of nucleotide diversity was observed among all individuals (0.018 ± 0.009) while the haplotype diversity was high (0.99 ± 0.001). Sequences generated for cytochrome b had 36 bp phylogenetically informative and this segment defined 32 haplotypes (Table 1). Also it resulted in low nucleotide diversity was found among individuals (0.0008 ± 0.0007) and haplotype diversity was 0.42 (± 0.01). Sequences generated for the nuclear protein coding recombination-activating gene 2 had 99 bp phylogenetically informative and defined 34 haplotypes (Table 1). Low nucleotide diversity was also found among individuals (0.0004 ± 0.0005) and high haplotype diversity (0.86 ± 0.02).

When we combined both mtDNA genes, we found 115 haplotypes, low nucleotide diversity (0.006 ± 0.003) and haplotype diversity was high (0.99 ± 0.001). The *Fst* pairwise between populations was low for all datasets and only the comparisons including Salvador population showed to be statistically significant (Table 2). Neutrality tests performed for all datasets, Tajima D and Fu's *Fs* were negative and non significant for all populations (Table 1).

Network haplotypes did not show any pattern of population structure. Further, each dataset presented different patterns. The CytB network had a starlike shape, where the most frequent haplotype is present in all populations with several localized lineages connected by short branches to a more common, widespread haplotype (Fig. 2). The great quantities of different haplotypes found for Dloop resulted in a network where there are a few haplotypes shared between different populations (Fig. 2). Rag 2 network had two most frequent haplotypes shared by all populations (Fig. 2). In the Dloop and Rag 2 networks we were able to include samples from Trindade Island (three and one

individuals, respectively). In the first network, one haplotype is shared with individuals from Florianópolis while the other two are exclusive. In the Rag 2 network we included only one individual, which showed to have one mutation step from an individual from Puerto Rico.

AMOVA results for all datasets and with both scenarios resulted that most of the genetic variability in *C. striatus* mtDNA corresponds to differences within populations, with values between 98-100%. Fixation indices over all populations (Φ_{st}) ranged from -0.005 to 0.006 but none of the values were statistically significant ($p > 0.05$; Table 2).

Pairwise F_{st} values for the three markers are shown in Table 3. Results from the mtDNA showed that with CytB, the population from Salvador is statistically different from the others, ranging from 0.06 to 0.16. With Dloop marker, Salvador was statistically different from all others with two exceptions: Arraial do Cabo and Florianópolis. Values ranged from 0.01 to 0.05. Results from the nuclear marker did not show significant differentiation among populations, as well as the mtDNA concatenated.

The model for Dloop was HKY + G; CytB was TIM + G. We used this information to run a Bayesian skyline plot. As the software BEAST 2.0 does not have the TIM + G model, we used HKY + G for both segments. We found a significant population size increase for the species around 80-100 thousand years ago (Fig. 3).

Discussion

The results from three molecular markers showed that most of the genetic differences are present among individuals from the same locality. This lack of phylogenetic structure among localities is supported by results found in the network analyses. The starlike shape found on CytB network suggests a relatively recent population expansion (Fig. 2). Dloop network showed a different pattern, with no population structure, where most of all haplotypes are unique and recent (singletons), which is also suggestive of recent population expansion. The nuclear marker (Rag 2) network presented two main haplotypes, shared by all populations (Fig. 2).

Differently from expected, the Orinoco and Amazon outflow barrier (Rocha, 2003; Floeter et al., 2008) seems not to have influenced the population structure of *C. striatus*, as can be seen in the network analyses. The lack of population structure can also be

checked with the low nucleotide diversity found for all populations with all datasets. In addition, the F_{st} between populations was low with few significant values (Table 3). The low values are common for marine fishes (Ward et al., 1994), however it is still unclear the reasons why these organisms show shallow populations histories, maybe due to historical bottlenecks, founder effect, natural selection (Grant and Bowen, 1998).

Analyses on Dloop and Rag 2 had higher haplotypes diversity (>0.7). This result together with the low nucleotide diversity found for this markers, suggests that this lineages had a recent population expansion after a long period of relatively small effective size, and because of the fast population size increase, the retention of mutations is favored (Grant and Bowen, 1998). Neutrality tests performed for all datasets, Tajima D and Fu's F_s were negative and non significant for all populations (Table 1), which also point to a recent population expansion.

Contrary to our prediction, the peripheral populations did not showed to have lower genetic diversity, the results being similar among all populations along the geographical range. Differences in biotic and abiotic characteristics found between the biogeographic provinces (Caribbean and Brazil), and along the Brazilian coast, such as the water temperature, could potentially act as a barrier responsible for differentiations between northeastern and southeastern regions, despite potential dispersive pelagic stage (Santos et al., 2006).

Chaetodon striatus larvae display a differentiated swimming ability prior to settlement, which may increase its dispersal potential (Stobutzki, 1998; Fisher, 2005; Leis and Yerman, 2012). This characteristic could favor a wider distribution of individuals, leading to population connectivity. In fact, recent phylogeographical analyses by Ludt et al. (2012) Lawton et al. (2011) and DiBattista et al. (2012) have been showing low population structure among very distant locations in the Indo-Pacific.

On the other hand, Puebla et al. (2012) found that there was no correlation between PLD and dispersal on five species of reef fishes in the Caribbean, including *C. capistratus* (*C. striatus* sister-species). When there is a lack of genetic structure in species with large geographic area this does not mean that necessary they disperse long distances once that it can be a result of stepping- stone dispersal (Puebla et al., 2012).

One molecular study has been done with *C. striatus*. Affonso and Galetti (2007) used RAPD marker to evaluate the genetic diversity in four localities along the

Brazilian coast and found that most of *C. striatus* genetic variation is intra-population. Our results in the AMOVA analyzes showed that 98-100% of the variance was within populations, however it was not significant. Also, the high variation intra-population is visible in the Dloop network, which present a mixed pattern of haplotypes from all populations, with high number of haplotypes found in each population.

With the Bayesian skyline plot analyses for all samples, it is possible to see an increase in the population size around 80-100 thousand years ago. These results are similar to previous studies on coral reef fish (Ludt et al., 2013). The fact that have had fluctuations on the sea level due to the Pleistocene glaciations, which directly had influence on the reef area size, probably explain the genetic patterns found for several reef fishes (Behling and Lichte 1997; Siddall et al., 2003; Ludt et al., 2012).

The spatial homogeneity of genetic variation found for *C. striatus* was found for other reef fishes, such as for *Paracirrhites arcatus* in a longitudinal gradient in the Pacific Ocean (Messmer et al., 2012). This species have similar results as *C. striatus* with the Dloop marker: high haplotype diversity, starlike shape haplotype network with no geographic grouping and low nucleotide diversity (<0.5). All this results indicate that both species had a recent population expansion.

The results present in here together with other studies (Liedke et al., in prep) encompassing biological features from *C. striatus* along its distribution (e.g. feeding behaviour) play a key role to develop conservation strategies.

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Legends

Table 1. Summary statistics of *C. striatus* with four datasets (CytB, Dloop, Rag 2 and mtDNA concatenated). n= number of individuals; hd=number of haplotypes; π = nucleotide diversity; h= haplotype diversity; Tajima's D and Fu's F statistics.

Table 2. Hierarchical analysis of molecular variance (AMOVA) was used to estimate levels of genetic differentiation among groups (fct), among populations within groups (fsc) and within populations (fst).

Table 3. Pairwise Fst values. On top, above diagonal values for Dloop, while CytB values are given below. On the bottom, above diagonal values are from mtDNA concatenated while Rag 2 values are given below.

Figure 1. Map with all sampling sites along *C. striatus* distribution range (gray line).

Figure 2. Median-joining haplotype network with three datasets a) Dloop; b) Rag 2 and c) CytB. Each color represent on population.

Figure 3. Bayesian skyline plot showing the effective population size fluctuation throughout time (heavy line, median estimation; thin lines, confidence interval).

Table 1. Summary statistics of *C. striatus* with four datasets (cytochrome b, Dloop, Rag 2 and mtDNA concatenated). n= number of individuals; hd=number of haplotypes; π = nucleotide diversity; h= haplotype diversity; Tajima's D and Fu's F statistics.

	n	hd	π	h	Tajima's D	Fs
<i>CytB</i>						
Puerto Rico	25	8	0.0008 (0.0007)	0.47 (0.12)	-2.27	-6.06
Tamandare	13	5	0.0005 (0.0006)	0.53 (0.16)	-1.65	-3.90
Salvador	19	6	0.0011 (0.0009)	0.71 (0.07)	-1.86	-5.37
Abrolhos	20	8	0.0012 (0.0009)	0.58 (0.12)	-2.04	-5.58
Arraial do Cabo	20	9	0.0012 (0.0009)	0.65 (0.12)	-1.91	-6.84
Florianópolis	19	8	0.0011 (0.0007)	0.61 (0.13)	-2.16	-6.01
Total	116	32	0.0008 (0.0007)	0.47 (0.12)	-1.98	-5.6
<i>Dloop</i>						
Puerto Rico	25	23	0.016 (0.009)	0.99 (0.01)	-1.89	-24.22
Tamandare	13	13	0.016 (0.009)	1.0 (0.03)	-1.49	-7.78
Salvador	20	19	0.022 (0.01)	0.99 (0.01)	-1.23	-1.02
Abrolhos	21	20	0.018 (0.01)	0.99 (0.01)	-1.56	-1.71
Arraial do Cabo	20	19	0.017 (0.009)	0.99 (0.01)	-1.67	-1.24
Florianópolis	18	15	0.019 (0.01)	0.98 (0.02)	-1.65	-1.18
total	117	111	0.018 (0.009)	0.99 (0.001)	-2.05	-1.55
<i>Rag 2</i>						
Puerto Rico	21	14	0.0006 (0.0003)	0.94 (0.03)	-1.65	-27
Tamandare	12	7	0.0002 (0.0003)	0.77 (0.12)	-1.14	-14
Salvador	17	8	0.0003 (0.0004)	0.84 (0.06)	-1.50	-14
Abrolhos	20	8	0.0005 (0.0005)	0.77 (0.08)	-1.87	-10
Arraial do Cabo	17	10	0.00	0.90 (0.04)	0.0	?
Florianópolis	17	11	0.0005 (0.0006)	0.91 (0.05)	-1.71	-21
Total	104	34	0.018 (0.009)	0.99 (0.001)	-1.12	-13

Table 1. continued.

Concatenated mtDNA	n	Hd	π	h	Tajima's D	FS
Porto Rico	25	25	0.005 (0.003)	1.0 (0.01)	-2.02	-23.33
Tamandare	13	13	0.006 (0.003)	1.0 (0.03)	-1.57	-6.98
Salvador	19	19	0.007 (0.004)	1.00 (0.01)	-1,35	-1.22
Abrolhos	20	20	0.007 (0.004)	1.00(0.01)	-1,74	-1.31
Arraial Cabo	20	19	0.006 (0.003)	0.99 (0.01)	-1,78	-11.26
Florianópolis	19	19	0.006 (0.003)	1.00 (0.01)	-1,89	-15.04
total	116	115	0.006 (0.003)	0.99 (0.001)	-2	-12

Table 2. Hierarchical analysis of molecular variance (AMOVA) was used to estimate levels of genetic differentiation among groups (Fct), among populations within groups (Fsc) and within populations (Fst).

(a) AMOVA results (PR) (TAM, SAL, ABR) (AC, FLO)				
	mtDNA			nuclear DNA
	CytB	Dloop	Concatenated	Rag 2
Among groups	0.52	-0.64	-0.06	1.46
Among populations within groups	-0.57	-0.7	0.6	-2.98
Within populations	100	99.9	100	100
Φ_{sc}	-0.005	0.006	0.006	-0.03
Φ_{st}	-0.0005	0.0006	-0.00007	-0.01
Φ_{ct}	0.005	-0.006	-0.006	0.01
(b) AMOVA results (PR, TAM, SAL, ABR) (AC, FLO)				
	mtDNA			nuclear DNA
	CytB	Dloop	Concatenated	Rag 2
Among groups	0.039	-0.46	-0.62	-0.62
Among populations within groups	-0.33	0.41	0.5	0.59
Within populations	99.94	100	100	100
Φ_{sc}	-0.003	0.004	0.005	0.005
Φ_{st}	0.0005	-0.0005	-0.0002	-0.0002
Φ_{ct}	0.003	0.004	-0.006	-0.006

Table 3. Pairwise Fst values. On top, above diagonal values for Dloop, while CytB values are given below. On the bottom, above diagonal values are from mtDNA concatenated while Rag 2 values are given below.

	P Rico	Taman	Salv	Abro	A Cabo	Floria
Puerto Rico	-	0.003	0.01*	-0.009	-0.001	-0.002
Tamandare	-0.01	-	0.05*	-0.02	0.01	-0.01
Salvador	0.06*	0.16*	-	0.03*	0.007	0.01
Abrolhos	0.001	0.01	0.12*	-	-0.001	-0.002
Arraial do Cabo	0.02	0.01	0.09*	0.02	-	0.004
Florianópolis	-0.002	0.01	0.08*	0.01	0.01	-
Puerto Rico		0.01	0.01	-0.004	0.001	0.002
Tamandare	-0.01		0.01	-0.01	0.01	-0.01
Salvador	-0.009	-0.07		0.01	- 0.003	0.005
Abrolhos	0.005	0.05	-0.02		0.01	0.01
Arraial do Cabo	-0.04	0.03	0.04	0.02		-0.002
Florianópolis	-0.02	-0.06	-0.03	-0.02	0.02	

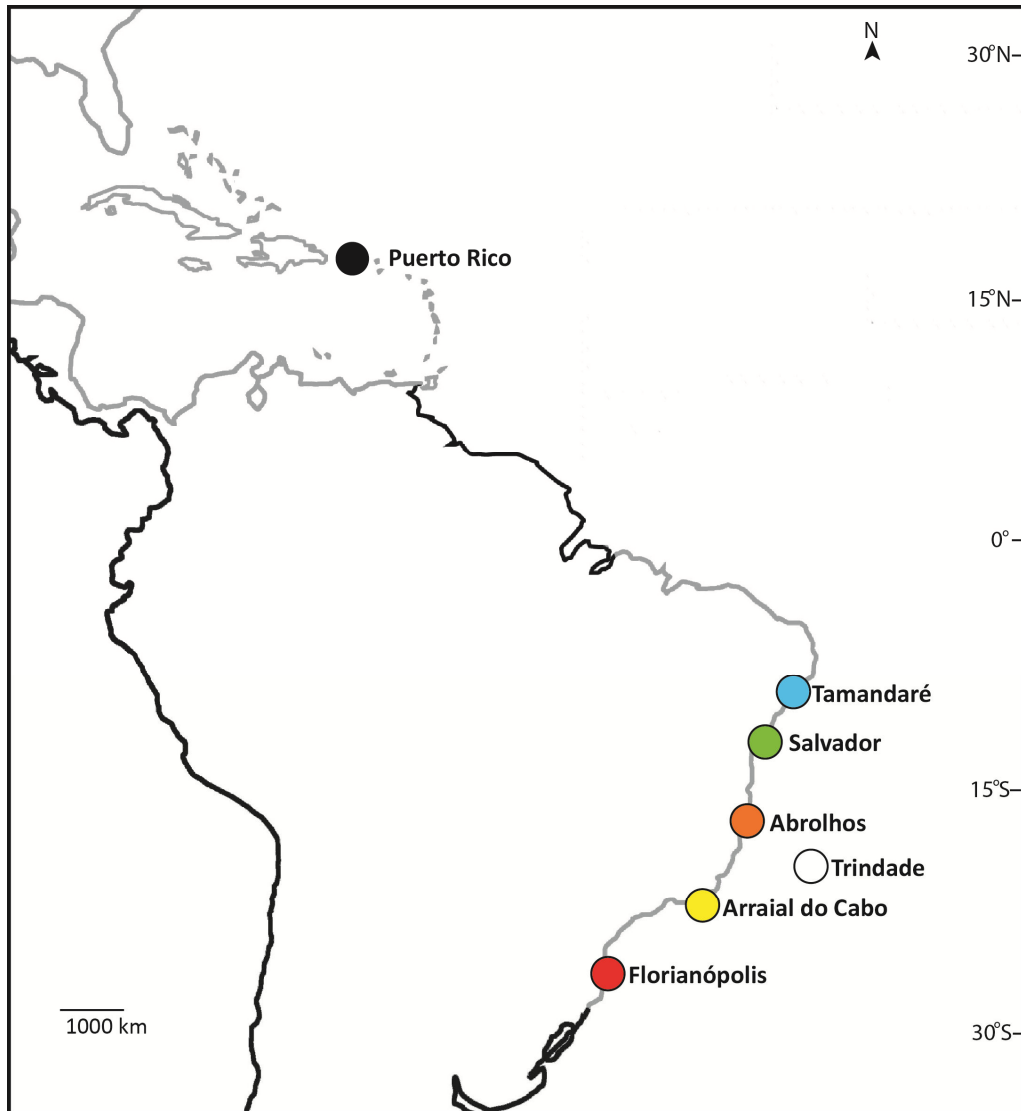


Figure 1. Map with all sampling sites along *Chaetodon striatus* distribution range (gray line).

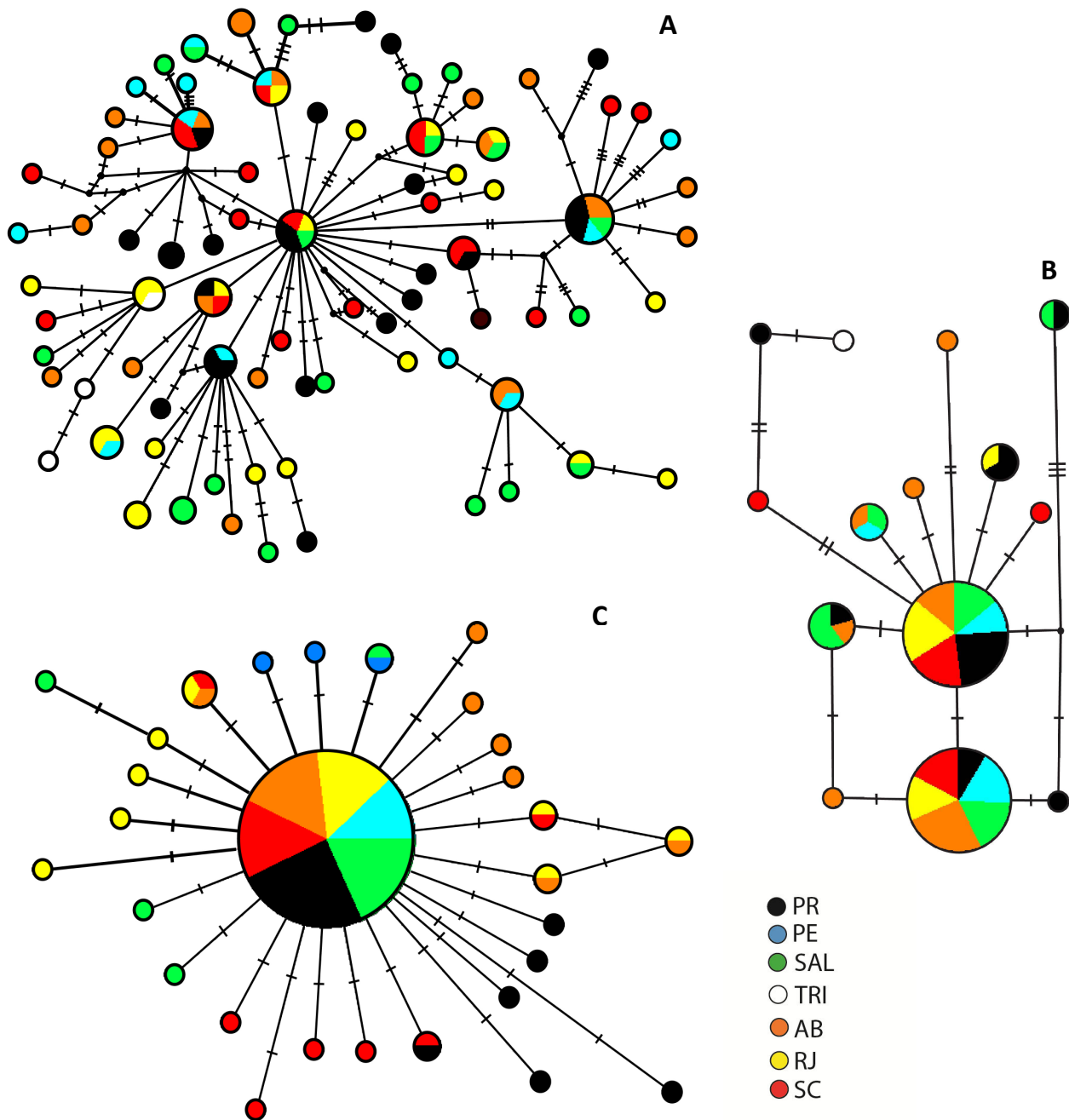


Figure 2. Median-joining haplotype network with three datasets a) Dloop; b) Rag 2 and c) CytB. Each color represent on population

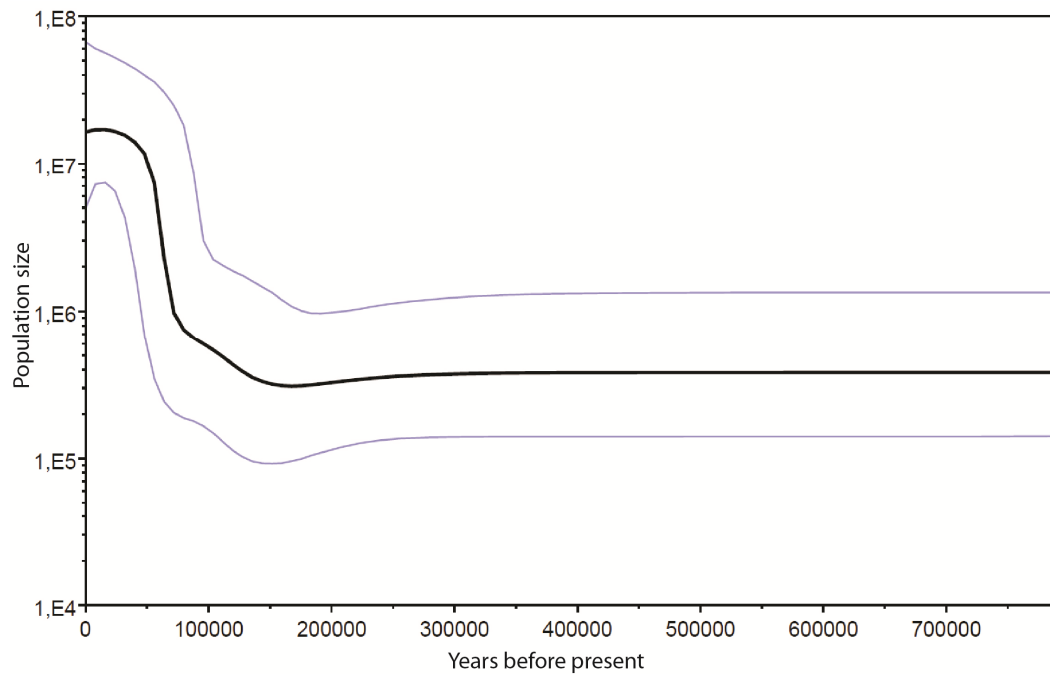
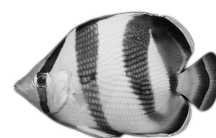


Figure 3. Bayesian skyline plot showing the effective population size fluctuation throughout time clade (heavy line, median estimation; thin lines, confidence interval).



Considerações Finais

No total foram observados em campo 649 indivíduos, analisados 1885 fotoquadrados, 214 estômagos, processados 100 amostras para razão RNA:DNA e 185 tecidos para análises genéticas (Tabela 2). Foram seqüenciadas 126 amostras com o marcador molecular para o mtDNA Dloop, 163 para o mtDNA cytB e 145 para o gene nuclear Rag 2.

Tabela 2. n° de observação em campo, n° de estômagos analisados, n° de amostras processadas para o cálculo da razão RNA:DNA e n° de tecidos processados para análises genéticas.

	n° observ. em campo	n° foto-quadrados	n° de estômagos	RNA: DNA	n° para genética
<i>C. striatus</i>					
Porto Rico	64	155	30	21	34
Tamandaré	78	210	17	-	17
Salvador	100	485	21	20	20
Guarapari	70	240	22	19	-
Abrolhos	100	295	20	-	20
Ilha de Trindade	-	-	17	-	
Arraial do Cabo	93	210	19	-	20
Florianópolis	63	155	20	20	21
<i>C. capistratus</i>					
Porto Rico	24	75	25	9	30
<i>C. humeralis</i>					
La Paz, México	57	60	23	11	23
TOTAL	649	1885	214	100	185

Os resultados obtidos no presente trabalho indicam que *Chaetodon striatus* é uma espécie bastante generalista quanto aos microhabitats utilizados para forrageamento e itens utilizados na alimentação. Além disso, essa espécie não apresenta estruturação genética ao longo da sua distribuição.

Mesmo encontrando diferenças significativas na média de mordidas por minuto em todas as localidades (ANOVA $p < 0,001$), as amostras das extremidades da distribuição tiveram resultados similares, apontando que a taxa de mordida não é influenciada pela composição do recife ou temperatura da água. A abundância dessa espécie não teve diferenças significativas entre as localidades amostradas.

A cobertura bentônica variou bastante entre as localidades amostradas (Fig. 4), porém sem padrão latitudinal ou direcionado por temperatura. No entanto, um declínio de antozoários em direção ao sul foi observado. Os microhabitats mais disponíveis foram: matriz

de algas epilitica (EAM) em cinco localidades (Porto Rico 36%, Abrolhos 44%, Guarapari 32%, Arraial do Cabo 24% e Florianópolis 42%), Anthozoa Zoantharia em Salvador (49%) e areia em Tamandaré (36%).

A porcentagem de mordidas em cada microhabitat também variou bastante entre as localidades. Em Porto Rico, 44,2% foi em EAM, em Tamandaré 37% das mordidas foram em alga foliosa, em Salvador 62,3% foi em Anthozoa Zoantharia, em Abrolhos 42% foi em Anthozoa Scleractinia, em Guarapari e Arraial do Cabo, 54,4% e 31,3% do em Anthozoa Octocorallia, respectivamente. Em Florianópolis, 40% das mordidas foram em alga crostosa (principalmente *Codium* spp.).

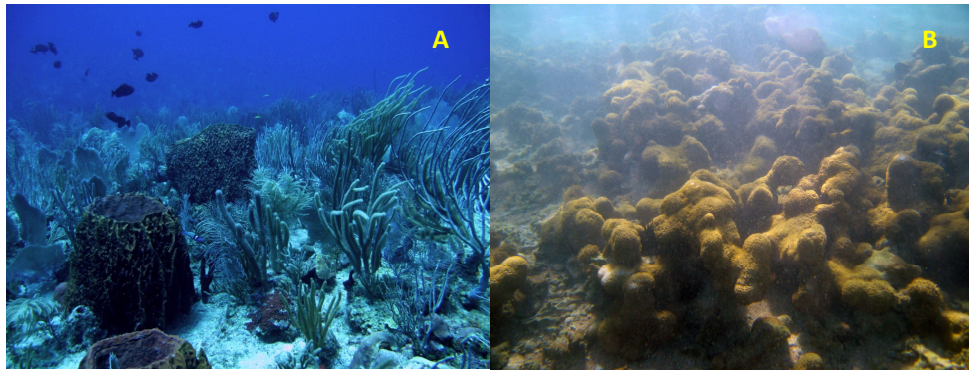


Figura 4. Ambiente recifal em A) Porto Rico e; B) Tamandaré, Pernambuco, Brasil.

Não foi encontrado nenhum padrão de seletividade por microhabitat utilizado para forrageamento por *Chaetodon striatus*. A maioria dos resultados do Índice de Eletividade - Ivlev ficou dentro dos valores esperados ao acaso, gerados pelo modelo nulo. Mesmo que o microhabitat EAM representou a maior parte da cobertura bentônica em cinco localidades, só foi selecionado em Porto Rico. Em Tamandaré observamos que os indivíduos selecionaram alga calcária articulada e rejeitaram algas crostosas. Em Salvador, observamos rejeição também em algas crostosas mais algas foliosas, que também foram rejeitados em Guarapari junto com alga calcária articulada e areia. Em Arraial do Cabo, os peixes rejeitaram porífera. Em Abrolhos e Florianópolis, todos os valores ficaram dentro do esperado ao acaso.

Análise do Conteúdo Estomacal

A análise de conteúdo estomacal resultou em 53 itens encontrados nos conteúdos estomacais das três espécies estudadas, sendo a maior parte composta por poliquetas, cnidários e artrópodes. Entre as populações amostradas encontramos entre 11 e 30 itens (Salvador e Porto Rico, respectivamente).

Chaetodon humeralis foi a única espécie que apresentou quantidade significativa de algas nos estômagos (Índice Alimentar - IAI 16,6%). Algumas espécies encontradas foram: *Bryopsis* sp., *Ceramium* sp., *Chondracanthus*, *Erythrotrichia carnea*, *Gelidium crinale*, *Hypnea* sp., *Jania* sp., *Naviculacea*, *Padina* sp., *Polysiphonia* sp., *Sargassum* sp., *Sargassum vulgares*, *Sphacelaria*.

Foram encontrados itens com baixo volume ou baixa frequência de ocorrência, resultando em baixo Índice Alimentar (IAI), porém são importantes complementos energéticos na dieta, como por exemplo pequenos crustáceos. Desova de moluscos e peixes também foram encontrados nos conteúdos. Como esses itens são consumidos oportunisticamente, de acordo com a disponibilidade no ambiente, podendo não ser considerado um item “regular” da dieta das populações. Portanto, para os resultados abaixo listado dos itens mais representativos IAI, as desovas foram excluídas.

Chaetodon striatus

Porto Rico: 56% do IAI é composto por poliquetas e 42,3% de Cnidaria Hexacoralia

Tamandaré: 84% do IAI é composto por poliquetas. [excluindo dados de ovos, que se incluídos representariam 75% IAI].

Salvador: 95% do IAI é Cnidaria Hexacorallia [36% Zoantharia]

Abrolhos: 54% do IAI é composto por Cnidaria Coralim/Scleractinia, 23% por poliquetas e 15% por outros cnidários.

Guarapari: 82% Cnidaria Hexacoralia (60% Zoantharia)

Trindade: 51,7% poliqueta, 29% Cnidaria Hexacoralia. Essa foi a única localidade brasileira onde encontramos Octocoralia (20% FO)

Arraial do Cabo: 74% Poliquetas e 14,5% Cnidaria Hexacoralia.

Florianópolis: 75,7% Cnidaria Hexacoralia (34,4% Coralim/Scle e 29,2% Actiniaria)

Chaetodon capistratus

Porto Rico: 74,5% Cnidaria (32,4% Coralim/Scle; 9,3 Octocoralia)

Chaetodon humeralis

La Paz: 40,8% Cnidaria, 40,1% poliquetas e 16,6% composto por algas.

Análise da razão RNA:DNA

A análise da condição nutricional, através da razão RNA:DNA de seis populações de peixes borboleta (uma população de *C. capistratus*, quatro populações de *C. striatus* e uma população de *C. humeralis*) resultou em valores similares. Esse resultado aponta que independentemente da composição da dieta e temperatura da água, todas as três espécies estão fisiologicamente bem adaptadas.

Resultados comparativos entre *C. capistratus* vs. *C. striatus*

A co-ocorrência de duas espécies de peixes-borboleta na mesma localidade é interessante, uma vez que pode existir partição de habitat ou diferenças no comportamento da espécie, para não haver sobreposição (p.ex. alimento, habitat) entre as espécies. Comparando resultados entre *Chaetodon capistratus* e *C. striatus*, verificamos que a primeira apresentou maior frequência de mordidas ($3,36 \pm 0,38$ EP) do que *C. striatus* ($1,38 \pm 0,16$ EP) (Teste T de Student $p < 0,05$). Estudos anteriores apontam que *C. capistratus* nas Ilhas de St Croix possuem frequência de mordidas de $5,9 \pm 1,9$ (EP) e $5,4 \pm 1,6$ (EP) (Birkeland e

Neudecker, 1981; Neudecker, 1985; respectivamente), revelando que nossos resultados foram levemente mais baixos.

Chaetodon capistratus apresentou maior densidade ($3,1 \pm 0,08$ EP) do que *C. striatus* ($1,5 \pm 0,7$ EP) (Teste T de Student $p < 0,05$) corroborando resultados encontrados por Pitts (1991). A baixa abundância de *C. striatus* no Caribe foi reportada por Neudecker (1985) nas Ilhas de St. Croix, na qual apontou como uma espécie rara.

A condição nutricional, revelada através da razão RNA:DNA entre ambas espécies foi estatisticamente igual, apontando que as espécies se mantêm fisiologicamente bem, mesmo com diferentes proporções de itens alimentares.

O comportamento de forrageio das duas espécies foi diferente. Enquanto *C. capistratus* forrageou em Anthozoa Scleractinia (31,4% das mordidas) e Octocorallia (33,1%) *C. striatus* forrageou 44% em EAM e 14,3% na areia. Mesmo EAM estando abundante para ambas as espécies, foi significativamente selecionado somente por *C. striatus*.

Como descrito por Pitts (1991), *C. capistratus* e *C. striatus* parecem ter uma partição de hábitat. Enquanto o *C. capistratus* se alimentava mais no centro das manchas de recife, *C. striatus* se mantinha mais nas bordas, junto com a interface do recife com areia. Nossos resultados mostram que *C. striatus* mordeu mais em microhabitats como areia, EAM e algas (69,5%), provavelmente em busca de invertebrados. Esse resultado foi corroborado pela análise de conteúdo estomacal, uma vez que 86% do volume encontrado foram de poliquetas e cnidários, que provavelmente vivem “escondidos” em algas e areia.

Chaetodon humeralis

Essa espécie apresentou a mais baixa frequência de mordidas por minuto entre todas estudadas, porém esse resultado é estatisticamente igual aos valores encontrados para *C. striatus*. Dados de abundância comparativos entre as três espécies revelam que *C. humeralis* é o menos abundante, tendo um indivíduo a cada 200m^2 . Por outro lado, a condição nutricional se manteve semelhante entre as três espécies do subgênero *Chaetodon*.

A cobertura bentônica encontrada em La Paz, Baja Califórnia Sur, México é bastante diferente das localidades amostradas no Atlântico Ocidental. Nessa localidade os microhabitats mais disponíveis foram: EAM (29,8%) e areia (30,3%).

Os microhabitats escolhidos para mordidas foram principalmente alga coriácea (40,8%) e areia (41,5%). Além desses dois microhabitats, EAM foi mordido com 16,3%. Nenhum microhabitat foi selecionado fora do esperado ao acaso, resultado gerado pelo modelo nulo.

Análises genéticas de *C. striatus*

As sequências de mtDNA do gene citocromo B (cytb) de seis populações ao longo da distribuição de *C. striatus* resultaram em 791 pares de base (pb), sendo 108 sítios variáveis e apenas 36 sítios parcimoniosamente informativos. Foram encontrados 32 haplótipos, que através do programa Network 4.6.1.1 foi feita uma rede de haplótipo utilizando o algoritmo “median-joining”. Através do resultado é possível verificar que existe um haplótipo compartilhado entre as seis populações (86 indivíduos) e diversos haplótipos com um passo de mutação.

O sequenciamento do mtDNA Dloop resultou em 397pb. Desses, 178 sítios variáveis e 93 parcimoniosamente informativos. No total, obtivemos 111 haplótipos, o que resultou em poucos haplótipos compartilhados.

O sequenciamento do gene nuclear Rag 2 resultou em 750pb, sendo 99pb parcimoniosamente informativos. Foram identificados 34 haplótipos, na qual a rede de haplótipo aponta para dois haplótipos presentes em todas as populações e bastante frequente.

Os índices de diversidade haplotípica (h_o) e nucleotídica (π) totais de *C. striatus* foram, para o Dloop $\pi = 0.018 \pm 0.009$ e $h_o = 0.99 \pm 0.0003$ e para o CytB $\pi = 0.0008 \pm 0.0007$ e $h_o = 0.47 \pm 0.12$ e para Rag2 $\pi = 0.018 \pm 0.009$ e $h_o = 0.99 \pm 0.001$

Com base nos resultados obtidos, verificamos que a rede de haplótipos gerada pelo mtDNA cytb mostra um padrão de estrela, na qual fortemente leva a interpretação que a espécie passou por uma expansão populacional recente. Ainda, a rede de haplótipos gerada com o mtDNA Dloop mostra um padrão na qual praticamente não existe compartilhamento de haplótipos, cada indivíduo possui um haplótipo com poucos passos de mutação do outro. Esse padrão corrobora a recente expansão populacional, uma vez que o marcador Dloop tem uma taxa de mutação maior que cytb, resultando em maior variabilidade.

Além disso, os resultados de diversidade nucleotídica e haplotípica para os marcadores moleculares seguem o padrão de baixos valores para diversidade nucleotídica e altos valores para diversidade haplotípica ($<0,5$ e $>0,5$, respectivamente). Esse resultado é característico de linhagens que sofreram expansão após um longo período com tamanho populacional efetivo pequeno, seguido por um rápido crescimento populacional (Grant e Bowen, 1998). Contudo, a população de Salvador possui baixo valor de diversidade nucleotídica e haplotípica, na qual pode indicar que a população passou por um gargalo populacional recente ou sofreu influência do efeito fundador (Grant e Bowen, 1998). Com esse cenário, verificamos que não existe estruturação populacional.

Para a Análise de Variância Molecular (AMOVA), realizamos com os três genes e dois agrupamentos: o primeiro entre as populações do subtropical (Arraial do Cabo e Florianópolis) e tropical (Porto Rico, Tamandaré, Salvador e Abrolhos) e um segundo agrupamento na qual separa o subtropical do tropical brasileiro (Tamandaré, Salvador e Abrolhos) e Caribe (Porto Rico). Todas as análises indicaram que mais de 98% das diferenças estão dentro de cada população, porém o Φ_{st} global não é significativo.

Segundo os valores de F_{st} par a par obtidos com *cytB*, *Dloop* e *Rag 2* as populações de *C. striatus* demonstram que não estão estruturadas. Poucos foram os resultados significativos, e todos ficaram abaixo de 0,2 indicando falta de estruturação populacional.

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